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CONTENTS

NUMBER 1, JANUARY, 1934

The Medicolegal Necropsy. A Symposium Held at the Milwaukee Meeting of the American Society of Clinical Pathologists, June, 1933. Introduction. FREDERIC E. SONDERN.....	1
The Medicolegal System of the United States. OSCAR T. SCHULTZ.....	7
The Medicolegal Necropsy. CHARLES NORRIS.....	24
Performing the Medicolegal Necropsy. A. V. ST. GEORGE.....	32
Pathological Anatomy of Death by Drowning. EDWARD L. MILOSLAVICH	42
Toxicology in the Medicolegal Necropsy. ALEXANDER O. GETTLER.....	50
Medical Examiners' Findings in Death from Shooting, Stabbing, Cutting, and Asphyxia. HARRISON S. MARTLAND.....	66
Appendix.....	161

NUMBER 2, MARCH, 1934

Recognition of the Allergic State by Tissue Examination, the Respiratory Tract, and the Nasal Sinuses. BERNHARD STEINBERG.....	169
The Specificity of the Test for Alcohol in Body Fluids. H. A. HEISE....	182
The Pathogenesis of Neutropenia. REGINA COOK BECK.....	189
The Differentiation and Standardization of Certain Streptococcus Toxins and Antitoxins by Means of the Skin Test. WALTER E. KING AND JOHN H. CONLIN.....	200
Histology after Thorium Dioxide (Thorotrast) in Hepatolienography. CARLO J. TRIPOLI.....	212
A Note on Acetone Insoluble Lipoids in Relation to Antigen for the Wassermann Reaction. JOHN A. KOLMER AND CAROLA E. RICHTER.....	235
Editorial.....	239
News and Notices.....	242

NUMBER 3, MAY, 1934

The Pathology of Psittacosis. Discussion with Case Report. ALVIN G. FOORD.....	247
A Study of Hyperpyrexia Reaction Following Intravenous Therapy. HORACE M. BANKS.....	260
Reticulocyte Counts in Healthy Children. EDWIN E. OSGOOD, RUSSELL L. BAKER, AND MABLE M. WILHELM.....	292

Carcinoids of the Appendix. A. V. ST. GEORGE.....	297
The Method of Diluting Antigen in Relation to the Wassermann Reaction. JOHN A. KOLMER.....	301
The Clinical and Pathological Findings Following Warfare Gassing. PHILIP B. MATZ.....	309
Editorial.....	312
News and Notices.....	314
Book Reviews.....	318

NUMBER 4, JULY, 1934

The Rôle of the Pathologist in the Cancer Problem. ALVIN G. FOORD...	321
Clinico-Pathological Relationship in Common Breast Lesions. FREDERICK H. LAMB.....	327
The Cause of Local Reactions Following the Administration of Staphylo- coccus Bacteriophage. WALTER E. KING, DAVID A. BOYD, JR., AND JOHN H. CONLIN.....	336
Blood Iodine Studies. IV. The Clinical Determination of Iodine in Blood, Urine, and Feces. FRANCIS J. PHILLIPS AND GEO. M. CURTIS.....	346
The Accuracy of Common Hemoglobin Methods. HOWARD L. ALT.....	354
The Presence of Arsenic in the Brain and Its Relation to Pericapillary Hemorrhages or So-called Acute Hemorrhagic Encephalitis. ARNOLD E. OSTERBERG AND JAMES W. KERNOHAN.....	362
Blood Dyscrasias, a Symptom Complex Rather than a Disease Entity. B. MARKOWITZ.....	370
Editorial.....	373
News and Notices.....	376
Book Reviews.....	379

NUMBER 5, SEPTEMBER, 1934

The One-Hour Two-Dose Dextrose Tolerance Test. WILLIAM G. EXTON AND ANTON R. ROSE.....	381
Some Medicolegal Aspects of Iso-Agglutinins. HERMAN A. HEISE.....	400
Preparation of Neurologic Material for Histologic Study. JAMES W. KERNOHAN.....	410
Further Studies in Antigen Emulsion Preparation for the Ball Test for Syphilis. B. S. KLINE.....	426
Cyanide Poisoning. A. O. GETTLER AND A. V. ST. GEORGE.....	429
Treatment of Malignant Neutropenia by Injection of Liver Extract. WALTER B. MARTIN.....	438
Editorial.....	446
News and Notices.....	450

NUMBER 6, NOVEMBER, 1934

The Etiology of Granulopenia (Agranulocytosis) with Particular Reference to Drugs Containing the Benzene Ring. ROY R. KRACKE AND FRANCIS P. PARKER.....	453
Diagnostic Methods in Amebiasis. Relative Value of Stool Culture as Compared with Other Methods. CARLO J. TRIPOLI AND MORRIS SHUSHAN.....	470
Rapid Pigment Appearance in Ohio Red Bellied Dace as Test for Intermedin. A. M. YOUNG.....	485
Endocarditis Caused by Diphtheroid Bacillus (Pleomorphic Streptococcus). ALVIN G. FOORD AND WILLARD J. STONE.....	492
Editorial.....	504
News and Notices, Minutes of the Thirteenth Annual Convention, Constitution and By-laws, List of Society Members.....	507
Index to Volume 4.....	543

THE MEDICOLEGAL NECROPSY

A Symposium Held at the Milwaukee Meeting of the
American Society of Clinical Pathologists,
June, 1933

INTRODUCTION

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This symposium embraces a series of articles of a definitely specialized character dealing with the determination of the cause of death in supposedly criminal cases as well as in some others which are also subject to review in courts of law. The result of such work often has a direct bearing on the conviction or acquittal of those accused of crime, and its methodical accuracy by specially qualified men with unusual experience has become a material aid in jurisprudence.

The "Coroners System" so universal in our country, means briefly an officer elected by his political party who appoints a local physician deputy to perform his medicolegal necropsies. His tenure of service is possibly but one term when he may be followed by a similar officer and deputy physician of other political affiliation. It stands to reason that no definitely expert service by specially qualified men with long experience can be developed in this way. With a full realization of the shortcomings of that system, the medical profession for years advocated its replacement by a Medical Examiner whose qualifications and service should be modelled on European usage.

The Medical Examiner is a civil service non-political appointment for life or at least a long period of years, the selection being made from a list of fundamentally qualified medical men. He organizes an office with such assistants as he may need and a laboratory with specialists in toxicology, histological pathology

and what not according to the extent of the service. A number of the large cities of the nation have now replaced the Coroner in this way with the result of a far more effective system as can easily be evaluated from the articles published herewith. Every city of sufficient size will do well to make the same change and if the size of the town does not justify these modern facilities for expert work such service can embrace a county, several counties or even a state.

Evolution in the service of the various Medical Examiners has demonstrated the fact that it is even difficult to secure properly qualified assistants for these services to say nothing of proper candidates for the positions of Medical Examiner. The various medical schools were appealed to for the establishment of post-graduate courses of special instruction for the purpose of better qualifying such candidates. The fall of 1933 saw the opening of the first unit of this kind, by the University and Bellevue Medical College of New York University. These courses in forensic medicine will include a series of lectures to undergraduates during the second semester of the fourth year and additional elective courses for them in the various branches of this specialty. Post-graduate courses will also be arranged to include comprehensive work for the qualification of Medical Examiners. These courses are now being established largely by the authors of the articles published herewith, all men in active service with a constant large amount of demonstrable material. Thus the undertaking will do much to advance forensic medicine and create needed specialists of this kind.

The careful post-mortem examination of the human body to learn the details of the causes of death in any case is without doubt one of the greatest factors in the progress of the accurate diagnosis of disease. This is so well evaluated by the pathologist Horst Oertel in a publication twenty years ago on the history of the autopsy in the development of scientific medicine, that a condensed review of his outline seems justified. It appears that Italy deserves the credit for the first step. While preceded by earlier workers of note, Morgagni in 1767 was really the first to correlate the change found at autopsy with symptoms of disease.

His was a pathologic-anatomic explanation of symptomatology rather than a system of pathology. Bichat in France soon after (1771-1802) based his theory of the *development* of disease on extensive studies of general anatomy in distinction to the establishment of the *seat* of disease by Morgagni. The Frenchmen Corvisart and Laennec, largely on the basis of Bichat's work saw in it the fundamentals of physical diagnosis. Subsequently these two men, though not actually the originators, perfected percussion and auscultation, Corvisart in 1808 and Laennec in 1815-1819. Austria became prominent soon after by the work of Rokitsansky (1804-1878) in Vienna. He traced the character and relationship of processes of disease by modern general pathology and particularly histologic pathology.

It is evident throughout in following the progress of medicine, that it went hand in hand with and was largely based on work on the cadaver, and nowhere is this more apparent than in the study of medical progress in Germany. During the first half of the nineteenth century under the speculative natural philosophy of Schelling, Germany remained far behind France, England, and Austria where serious efforts to overcome such purely speculative ideas and systems was making rapid headway. While Rokitsansky in Austria and Corvisart and Laennec in France were establishing pathology as the foundation for diagnosis, Germany was still in the grasp of battling schools and systems such as homeopathy of Hahnemann, polypragmasia, Rademacher's system, Priessnitz' system, Mesmerism, etc. An illustration of what was taught in pathology can be seen from a translation of few sentences from a syllabus entitled: *The Natural Families of Diseases* (Berlin 1851).

Disease has, although it represents a process of death, an organic combination of several actions, which develop from the innermost part of one nucleus. This nucleus is its principle. Disease grows from this nucleus to a system of actions. In this system the nucleus represents the fundamental action, the stem of the disease, from which extend many branch actions as for instance constipation, colic, vomiting, fever, etc.

It becomes more ridiculous as it continues.

Virchow, that great father of pathologic anatomy in Germany

said at the beginning of his outstanding career in 1854, "German medicine on account of its views and dissenting schools has been the laughing stock of the world." Definite as was the lack of progress in medicine in Germany during the first half of the nineteenth century in which period pathology was completely neglected, just so pronounced was the advance to a position of greatness and leadership during the second half of that century in which period Virchow did more to establish pathologic anatomy as the foundation of clinical medicine than any other man before or since. He taught and demonstrated at the autopsy table with a system, perseverance and force never approached before, that every disease represented an anatomically localized process and that therefore the goal of all pathologic conceptions must be objective knowledge of and location of the diseased processes—no speculative idea.

Upward of twenty years ago Richard Cabot of Boston rather startled the profession in his arguments favoring post-mortem examinations, with the publication of the percentages of correct clinical diagnosis of what appeared to be the most important lesion revealed by 3,000 autopsies. Only a few of the more evident diseases exceeded 75 percent in the hands of an experienced diagnostician, many important diseases falling below 50 percent in recognition and some even below 25 percent.

About the same time, namely twenty years ago, Corwin in a statistical study of the percentages of autopsies obtained on persons dying in hospitals in the United States compared with those in other countries, demonstrated the striking fact that the figures in our largest hospitals were much lower than those in similar institutions elsewhere. The average percentage of autopsies in the stated number of the largest hospitals in the various countries was as follows: United States, seventeen hospitals average 22 percent. Canada, three hospitals average 60 percent. England and Scotland, five hospitals average 76 percent. Germany and Austria, eight hospitals average 90 percent. Of course these figures have undergone much change in these last two decades and while there has been a very decided improvement in our own figures they are still not comparable with those of the other coun-

tries. The reasons for our difficulty in obtaining autopsies on persons dying in hospitals are, the claims of the anatomical departments of our medical schools for unclaimed cadavers, adverse public opinion to autopsies, the autopsy law, and the undertaker's objection to an autopsy.

The claims of the anatomical departments of the medical schools on unclaimed cadavers for purposes of dissection by students and others are regulated by law which only too frequently robs the clinical and pathological departments of the hospital of the benefits of post-mortem study in these cases. This problem has not been solved as yet and unfortunately the Continental system of clearing all anatomical material through the pathological department would be difficult if at all possible to introduce.

Adverse public opinion to autopsies has been considerably reduced in recent years by the increase in public health education by means of lectures, radio addresses, magazines and newspapers with the result that religious and other scruples are being overcome. The tact and sympathy of the hospital official whose task it is to obtain consent to an autopsy is most important and now generally realized.

The laws concerning necropsies vary in the different states of the Union and as a whole obstruct rather than facilitate securing post-mortem examinations. For example, the former law in New York was such that almost anyone could claim a body, and could prevent an autopsy by withholding consent. It was a common practice for undertakers to claim bodies, prevent an autopsy and then seek next of kin more diligently perhaps than the hospital did. Largely through the efforts of the New York Academy of Medicine the law was recently changed and now prevents this and other evils, but it is not yet a model law. The matter of framing a uniform model law and having it enacted by the various states in the interest of medical science is one which deserves serious attention on the part of medical educators and the profession generally.

The undertaker's objection was found to be perhaps the most important obstacle to an autopsy, at least in the City of New York, by his quiet firm advice against it to members of the family

arranging for the funeral. This attitude can easily be understood when realizing the relatively large number of post-mortem examinations formerly made by junior hospital physicians as well as the frequent disregard even by the experienced pathologist of the need of leaving intact structures required for proper and uncomplicated embalming. Realizing the importance of securing the good will of the undertakers to eliminate their objections, a joint committee was formed to represent the New York Academy of Medicine, the New York Pathological Society, and the Metropolitan Funeral Directors' Association. This Committee prepared a report the value of which is such that it has been included in the papers published in this monograph. It has also been printed in display form and is posted on the walls of every autopsy room in New York. A Subcommittee on Grievances was also created and is subject to call at any time to adjudicate complaints by a pathologist or a funeral director as the case may be. While such grievances were rather frequent at first there has not been a single one in the last six months. The benefits which have come to both the pathologists and the funeral directors are such that a similar arrangement in other large centres is urged.

THE MEDICOLEGAL SYSTEM OF THE UNITED STATES

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INTRODUCTION

It is perhaps fitting that a discussion of the medicolegal system of the United States should introduce a symposium on the medicolegal necropsy. Such a discussion will have to deal with the system that prevails throughout the greater part of the country. That system is the one with which most of you, as pathologists, come in contact. The prevailing system leaves much to be desired. What I shall discuss, therefore, forms a contrasting background for what is to follow by those who work under a better system that is in use in only a few localities.

I am not sure that any suggestions that I may make for improving the status of legal medicine in the United States will have the complete approval of you who, as pathologists and experts in laboratory medicine, may receive some financial gain from your participation in the workings of the existing system. I maintain the thesis that the important subdivision of legal medicine which has to do with "the application of expert medical knowledge to the needs of law and justice" (Oertel²) is just as much a function of government as is the administration of justice itself. A system that would make available to the administration of justice the best type of medical service would necessitate some form of fulltime or parttime service that would probably take away from some of you some of the odd jobs that you perform under the present system. But I am sure that you will be willing to subordinate such opportunities for minor personal financial gains to the greater good of society at large.

APPLICATION OF MEDICINE TO LAW

The application of medical knowledge to law occurs along several lines that have been left more or less distinct under the American system. First comes the investigation of those deaths into whose causation the element of criminality or accident may have entered. Of equal importance are questions involving the mental status or legal responsibility of one accused of crime. Fatal and nonfatal injuries to the person through accident or casualty present many perplexing problems that find their way into the civil courts. A final application of medicine to law is that which concerns the use of medical and other sciences in the detection of crime and in the apprehension and identification of suspects.

These various legal aspects of medicine often involve the manner of presentation of medical facts and opinions to the legal tribunal. This carries over into the field of expert medical testimony, an important subdivision of legal medicine that can receive no more than this brief mention here. Medicolegal psychiatry concerns the pathologist only in so far as neuropathologic or laboratory questions may be involved. The pathologist may occasionally be called upon in personal injury cases, and his aid is sometimes sought by the police, but the most important medicolegal activities of the pathologist come into play in connection with the first of the applications of medicine to law mentioned above, namely, the investigation of supposedly criminal or accidental deaths. It is this aspect of our medico-legal system that I propose to discuss more fully.

THE CORONER

Except in a very limited number of jurisdictions, the investigation of deaths that require the attention of an agency of government is made by the office of coroner. It is not necessary to trace the history of this ancient and honorable office, save to mention that it was transplanted from England to the original colonies. From them it was transferred to the states. In twenty-four of the states the office is unfortunately provided for

by the state constitution. I say unfortunately, because the mere mention in the constitution that there shall be such an office as that of coroner makes necessary a constitutional amendment if the office is to be replaced by the more efficient system under which others taking part in this symposium work.

The duties of coroners are prescribed by statute. In most states these laws were framed when a rural type of civilization prevailed and the population was widely scattered. The condensation of population into large industrial urban and suburban communities has brought about conditions that did not exist and that could not be foreseen when the original laws were drawn. The statutes relating to coroners have undergone no essential modifications that would better fit them to meet modern conditions.

No one will question the need of official inquiry into the cause and manner of certain kinds of deaths. Suspected homicide certainly belongs in this category. If death is presumably due to suicide, the coroner must act because death due to the unlawful act of another must be excluded. In most jurisdictions deaths due to accident or casualty must also be investigated by the coroner, because criminal or negligent actions of others may be involved. The coroner's investigation assumes great importance in suicidal and accidental deaths, not only because of their possible criminal or negligent content, but because important civil questions relative to workmen's compensation and accident and life insurance may be involved. A previous analysis^{3, 4, 5} of the kinds of deaths requiring such official action as is here discussed revealed that in five populous jurisdictions homicidal deaths constituted only 1.6 to 6.5 per cent of the total, suicide only 5.4 to 9.5 per cent, and casualty from 30.3 to 40.7 per cent. In four of the jurisdictions, all forms of violent death constituted only 39.6 to 49.1 per cent of the total; in the fifth jurisdiction the figure was 56.7 per cent.

The physician will be quick to recognize still another group of cases in which some sort of official action is necessary. The layman and the lawyer give little thought to this group, although their importance is evident from the fact that they made up more

than half of the totals in the jurisdictions whose figures were analyzed. These are the deaths of persons who have not been attended by a physician and who are dead or dying when the physician arrives. The physician has no right to issue the death certificate in such a death, although he is frequently importuned to assume this responsibility and sometimes does assume it. The responsibility of issuing or authorizing a death certificate rests with an agency of government, which agency in most localities is the office of coroner.

THE CORONER'S NECROPSY

In no instance can the cause and manner of death be determined with the scientific accuracy that should be required by law without a necropsy. Many, and especially many physicians, seem to think that the coroner has unlimited authority in the matter of performing or authorizing a necropsy. It might be a good thing for the future of medicine and of humanity if coroners did have such broad authority, provided it were intelligently used. Vital statistics as they relate to causes of death and to the incidence of disease would certainly be more accurate than they are at present. But the coroner's office is not a diagnostic service, and in the determination of the cause of nonviolent deaths the activities of the office are restricted.

In the performance of necropsies the coroner is restricted to those instances in which death is presumably due to violence. He can presume that death is due to violence until proved to the contrary by necropsy. As a matter of fact, in many jurisdictions the coroner does necropsies in nonviolent deaths upon the basis of such an assumption. In most of the legal actions brought against coroners for the illegal performance of necropsies and in which the verdict has been against the coroner, the adverse judgement has been based upon the fact that he did not have sufficient reason to warrant him in assuming that death was due to violence. Apparently there must not be a merely negative presumption that death may have been due to violence until proved otherwise by necropsy, but there must be strongly positive presumption that death was due to violence before the necropsy is done.

The trend of legal opinion, in those instances in which higher courts have had to determine whether the coroner had overstepped his authority, seems to consider the coroner's necropsy a part of the inquest, and not something apart from the latter which might lead to an inquest. Such an interpretation of the statutes would result in extreme restriction of the authority of the coroner in some jurisdictions. I am referring now to the magisterial or quasi-judicial inquiry known as the inquest, as opposed to the mere preliminary investigation or view of the dead body. In only eight states and the District of Columbia are the statutes so worded as to authorize an inquest if the deceased was untended by a physician and the cause of death is unknown. In four states deaths due to casualty or accident are subject to inquest, but deaths due to unknown causes are excluded. In the remaining states, excluding the New England states that use the medical examiner system, the holding of inquests is restricted to those deaths that are presumably due to "unlawful means" or to the "criminal or unlawful act of another." Perhaps in the small number of states of this group, whose statutes include the words "default or negligence of another," this phraseology might be construed to include deaths due to casualty.

THE CORONER'S INQUEST

The restrictions that hedge about the holding of inquests seriously limit the authority of the coroner. But even in those instances in which the statutes of every state authorize an inquest, namely deaths due to suspected murder or manslaughter, the inquest would appear to be a most futile and inutile procedure. It is futile because of the lack of training in law or criminology of the average coroner and because of the character of the average coroner's jury. It is useless because the kind of investigation that the coroner's inquest is belongs more properly in the hands of the prosecuting attorney, who is responsible for the conduct of the criminal legal procedure that may arise out of the investigation. Emphatic attention has been called to the useless, and often harmful, character of the coroner's inquest by Joseph Du Vivier,¹

a former assistant district attorney for New York County, who said:

The coroner does nothing that must not be done over again. No reliance can be placed on anything that he has done, nor can he be trusted to do anything right. Every case in which there may be criminal responsibility must be watched.

Many changes are necessary if the coroner's office is to become the efficient, modern medicolegal agency that it should be in our system of judicial administration. One of the most fundamental and important changes is the abolition of the coroner's inquest as a function of the coroner. By making the prosecuting or state's attorney responsible for the inquisition that the coroner now conducts, not only would duplication of work be avoided and better work be done in criminal cases, but the costs of jurors' and witnesses' fees entailed by the coroner's inquest would be eliminated.

CAUSES OF POOR FUNCTIONING OF THE CORONER'S OFFICE

What has been said is condemnatory of the office of coroner as an agency of government. Individual coroners differ in intelligence, in general ability, and in the efficiency with which they perform the duties of their office. But chief condemnation falls not upon the individual who may be coroner, but upon the office itself as a part of the governmental machinery. The faults of the office are largely those of the laws under which it works. The office is an elective one. It is an obscure political office that is usually passed out to some minor light in the partisan political organization. In some jurisdictions it does not have even this doubtful distinction and may be had for the asking by one who has not yet attracted political attention. The tenure of office is short. Although the office has duties of a highly technical or specialized medical and legal character, in most states no qualifications are required of aspirants for the office.

The authority of the coroner in the performance of his duties is, as has already been indicated, poorly defined. The laws relating to the office have been made antiquated by changed conditions. To quote Du Vivier again:

A dispassionate study of the office leads one to the inevitable conclusion that it is an institution of government wholly unsuited to the needs of the day.

Perhaps the most fundamental problems connected with the functions of the coroner's office arise out of the fact that it is of variable importance in different states and in different units of the same state, a fact that the laws relating to the office fail to recognize. In 1932 there were only seven inquest cases in each of two Illinois counties. In 1930 there were 4,098 inquest cases in Cook County of the same state. It is obvious that the latter jurisdiction makes much heavier demands of its coroner's office than does either of the other two counties. However one or a few homicides in a rural community may be just as disturbing to the social structure and may demand just as careful work in a just administration of justice as a larger number of similar cases in a metropolitan area. Until this difference dependent upon population is recognized by society, the important medicolegal work of a congested urban jurisdiction will continue to be hampered by laws that function none too well even in a sparsely settled rural county. In the failure to recognize the relation of density of population to the work of the coroner the American governmental system reveals its failure to recognize an important feature of the English coroner's system. The latter has long made a distinction between coroners of counties, coroners of boroughs, and coroners of the city of London.

IS THE CORONER'S OFFICE NECESSARY?

There would be little logic in criticizing the coroner's office as a medicolegal agency if nothing better could be or had been devised to take its place. We have lived so long with the office that some seem to think that life and government without the office are impossible. But a number of states get along as well, but not necessarily any better, without county coroners than do many states with coroners. In seven states the office of coroner does not exist, justices of the peace performing the duties usually assigned to coroners. In an eighth state, with an elective coroner system, the statutes provide that justices of the peace may view bodies and hold inquests. What may be the advantage of substituting the justice of the peace for the coroner, other than the elimination of a minor elective official, is not apparent. It would appear to be an admission that the work done by coroners in

other states is held to be of relatively little importance in those states where the justice of the peace does such work.

In Nebraska, the county attorney performs all the duties that had been enjoined by law upon the county coroner previous to 1917. In the state of Washington, the prosecuting attorney acts as coroner in counties with a population of less than 40,000. This is a distinct improvement over the usual coroner system. It places the important legal duties of the coroner's office with an official trained in the law. The medical duties are performed by physicians named by the county or prosecuting attorney, just as in most states a physician is selected by the coroner. The system is faulty in that it prescribes no qualifications for the examining physicians and provides for no definite period of service.

REFORM OF THE CORONER'S OFFICE

Reform of the American medicolegal system, in those of its aspects that have to do with the investigation of deaths, with the application of psychiatry to law, and with the presentation of expert medical opinion to tribunals, is imperative if the people of the United States are to have a system of judicial administration comparable in high character of work to that of the countries of continental Europe, of Scotland, of Egypt, of Japan, and of a number of South American countries. In so far as the work usually done by coroners is concerned, the way to better service has already been put into effect in a number of jurisdictions. The method of procedure in use in Nebraska and the state of Washington lacks only provisions for the appointment of qualified physicians and their continued service under conditions that will free them of political pressure to make that method practically identical with the medical examiner system that functions so excellently in Suffolk County (Boston), Massachusetts, New York City, and Essex County (Newark), New Jersey, and that does somewhat less better work in the rest of the state of Massachusetts and in the other New England states.

Under the medical examiner system, the examiner is an appointive official. He serves either continuously under civil service, as in New York City, or for a stated period of years, as in the other

jurisdictions. If appointed for a term of years he is reappointed repeatedly. The result is that in New York City, Suffolk County, Massachusetts, and Essex County, New Jersey, the medical examiners have served continuously for many years. Their appointment in the beginning having been based upon a knowledge of pathology, the value of their services to their communities through increasing experience gained by continued work in a technical field should be obvious. If the medical examiner's investigation brings to light anything suggesting criminality or negligence, he reports his findings directly to the district or prosecuting attorney, who makes the necessary further legal investigation of the case. Although the examiner reports his findings in criminal cases to the prosecutor, he is not influenced by political obligations to the latter, since the appointment of the medical examiner is not made by the prosecutor. The medical examiner system has the patent advantages of a simpler and more direct method of procedure in criminal cases and of expert medical service in all deaths that must be referred to some agency of government. It has the added advantage of modern laws drawn to meet contemporary conditions.

It is difficult to see how the coroner's office may be reformed short of complete abolition. The most fundamental reform that is necessary in the interest of better public service is the abolition of the inquest and the transfer of the coroner's inquisitorial functions to the public prosecutor. Such a change would leave to the coroner only nonviolent deaths. The investigation of these is primarily a medical problem and should be made by medical experts. Action by a coroner would not be necessary in such cases. The medical expert could transmit his findings directly to compensation boards, registrars of vital statistics, health departments, or any other agency entitled to know the facts. Abolition of the inquest as a duty of the coroner therefore logically leads to abolition of the office itself and to the replacement of the coroner by the medical examiner.

The superiority of the medical examiner system in those populous communities where the system has been tried is without question. But this system has not yet been as well adapted to

the needs of less densely populated parts of the state as should be the case. Massachusetts abolished the office of coroner and substituted that of medical examiner in 1877. The two examiners of Suffolk County are on a fulltime, salaried basis. In the rest of the state the examiners are on a fee basis. Their number varies from one to eleven for each of the thirteen counties outside of Suffolk. This state-wide medical examiner system is a decided improvement over the antiquated coroner system, but it is not as well adapted to modern conditions as it might be, because at the time of its adoption the important rôle of automobile transportation in American life could not be foreseen.

A modernized state medical examiner system should disregard county limits, if this is constitutionally possible. Each medical examiner district of the less densely populated portions of the state should consist of several counties, the size of the district being determined by population, which is the factor that governs the amount of work that must be done, and by local transportation facilities. The cost of maintenance of such a medical examiner district could be prorated among the counties constituting the district. In a number of states two or more counties may unite in the construction and maintenance of a sanatorium for the treatment of tuberculosis. The cost of the care of indigent patients in the hospital of the state University's medical school is charged against the county from which the patient comes. A state medical examiner system composed of districts such as have been suggested would give to all parts of the state a much higher type of expert service than is possible under any other system.

To provide each examiner district of the state with all the laboratory facilities that might at times be necessary for thorough and scientific medicolegal work would probably entail a greater expenditure than the volume of work to be done would warrant. In a state medical examiner system in which the local districts are in charge of appointed and reasonably well trained pathologists, there should be a central state agency, to which local examiners might refer such materials as require microscopic, chemical, or other scientific investigation and such problems as

require highly expert opinion for their solution. The creation of such an agency in each state, for which the name medicolegal institute might be adopted, has been recommended by the American Medical Association and the American Bar Association.

Back of whatever medicolegal system that might be devised must be laws that clearly define the duties that are to be performed under the system and the authority for the performance of the prescribed duties. Such statutory definition must be made in the light of the needs of society as it is constituted at the present day.

PERMISSION FOR NECROPSY

I have been asked to suggest a form for a fool-proof necropsy permit. I am afraid that it will be impossible to devise a fool-proof permit until such time as our social organization reaches the millennial state when fools are no longer bred or developed. A wise man has said that any fool can start a civil law suit if he can get a knave of a lawyer to bring the suit. Whether he will win the suit is another question. But so long as fools and knaves exist, suits will be brought for the alleged illegal or unauthorized performance of necropsies.

Most of the suits of this kind that have been brought have been against coroners. The coroner needs no expressed permission to perform or authorize a necropsy. The statutes define the conditions under which he may exercise this important medical function. As has already been pointed out, difficulties arise because the statutes are not precise enough in their definition of authority. The coroner may find himself brought sharply to a halt by a court decision to the effect that he has overstepped the bounds of his statutory authority. The matter of legal permission for a coroner's necropsy requires little further discussion here. The question is one of what the coroner may do under the written laws relating to his office. He may not be able to find the answer to the question except through a court decision. The liability rests with the coroner who authorizes the necropsy, rather than with the physician who does the work under the orders of the coroner.

For the performance of a necropsy by another than the coroner, when no medicolegal problem is involved, permission is necessary. It is to be noted, however, that in only one state, Connecticut, is it necessary that the permission be in written form. In this connection it may also be noted that in one of the most important University teaching hospitals in the country, which has the high permission necropsy percentage common to such hospitals, no relative is asked to sign any form of permit. The attending physician signs a printed form affirming that he has received permission for necropsy from the person whose name and relationship to the deceased are written into the form. In the twenty-two years during which this method has been in vogue in that hospital, no difficulties have arisen.

Most hospitals, as a matter of protection, require that the permission for necropsy be in writing and be signed by a responsible person. While such a permit may not actually be necessary, there can be no question but that it protects the hospital against knavery and mendacity. It may not prevent the bringing of a law suit, but it may be the main factor in preventing the loss of the suit. When difficulties arise over the performance of a necropsy, permission having been actually given, they may arise out of any one of several factors.

The allegation may be made that permission was given by one not legally entitled to grant it. The person who may give permission to hold a necropsy is the one entitled by law to the custody of the body. In general, this is the surviving spouse or the next of kin. If there are several relatives of equal degrees of kinship, as two parents or several brothers and sisters, it would not appear necessary to have the consent of all such kin. The right of custody carries with it the duty of legal disposal. The relative who accepts the custody of the body and the responsibility of sepulture would appear to be the one who should sign the permit. An adult son of the deceased may set up the claim that permission for necropsy given by another son or daughter of the deceased is not valid, because it was not made with the consent of the complainant. In such instances, the controversy would appear to be among the kin themselves, rather than between a next of kin and the hospital or pathologist.

Suits have been brought on the ground that the person who granted permission and had a legal right so to do alleges that he did not know what he was signing when he affixed his signature to an autopsy permit. Such a claim would probably have little standing in a court of law if the hospital acted in good faith in the performance of a necropsy.

A more reasonable ground for complaint may arise out of the claim that the hospital or pathologist did not act in good faith when the permission for necropsy was obtained. It may be claimed that the autopsy permit was granted under a promise that the examination would be limited to such as might be made through a small incision. Interns have been known to obtain permission for necropsy under such a promise. The signature is affixed to the usual form, which places no restrictions upon the examination to be made. If the pathologist now proceeds with a complete necropsy, as he has the undoubted right to do under the signed permit brought to him, the promise made by the intern, as a representative of the hospital, has not been kept. A charge of bad faith is ethically justifiable. Certainly no blame attaches to the pathologist, who has acted in good faith. The liability of the hospital would probably have to be determined in each individual case, upon the merits of the case.

When the permission for necropsy has been obtained in an honest and straightforward manner and the necropsy is done with the care and decency that it deserves, few controversies will arise over alleged unauthorized autopsies. If chicanery or dishonesty has entered into the obtaining of the permit, or if the necropsy has been improperly done, there would appear to be some cause for complaint.

REMOVAL OF ORGANS AT NECROPSY

Pathologists do not hesitate to retain organs removed at necropsy. Any proper teaching of gross pathology is inconceivable if the changes caused by disease are not illustrated by organs removed at necropsy. But the right to retain such organs may be questioned. Upon this point Weinmann⁶ has expressed the following opinion

If a physician performs an autopsy which he is legally authorized to do, either by the consent of the persons entitled to sepulture or by virtue of statutory authority, including direction from a coroner, such physician cannot legally remove and retain portions of the body unless such procedure is essential for determining the cause of death or for the determination of some other reason for which the autopsy was ordered or permitted.

Every pathologist will probably have to admit that he has retained organs for teaching or museum purposes, rather than that their retention was essential to the determination of the cause of death or the establishment of the nature of the disease process present. Permission for necropsy in non-medicolegal cases is usually given not merely for the purpose of determining the cause of death, but to elucidate the nature of disease and to further medical knowledge. The removal of organs would therefore appear to be permissible upon the ground that its purpose is the "determination of some other reason for which the autopsy was ordered or permitted" (Weinmann). The matter could be very easily covered by a clause in the necropsy permit authorizing the pathologist to retain such organs as he might deem necessary for further study. In general, however, the form used to grant permission for necropsy should contain as few qualifying clauses as possible.

DISPOSITION OF PARTS REMOVED FROM THE LIVING BODY

One of the essentials of an approved hospital, required by both the American Medical Association and the American College of Surgeons, is that all tissues removed by surgical operation must be sent to the laboratory for the proper examination. Hospitals seem to take it for granted that the disposal of removed tissues or parts in conformity with this requirement is a right of the hospital. Controversies sometimes arise between the hospital pathologist, who looks upon such surgical material as the property of the hospital, and the surgeon, who may insist that the specimen belongs to him.

The mere fact of the surgical removal of parts of the body does not make them the property of the hospital, the pathologist, or

the surgeon. Under the common law the living person from whom such parts are removed or his legal representative has the right to direct the disposal of tissues, organs or parts removed from the body. In four states, New York, North Dakota, Oklahoma, and South Dakota, this right has been enacted into statute law. In the three last named states the statutes are so worded as to make the laws relating to the dead human body applicable to removed parts. Enforced to the letter, these statutes might make necessary a burial permit for a pair of removed tonsils or a transportation permit for a specimen sent out of the state for histologic examination.

Few legal controversies have arisen out of this right of a person to direct the disposal of removed parts. But the element of trouble exists if a patient should choose to make trouble. Possible difficulties could be very easily obviated by including in the permit for operation, which most hospitals or surgeons require, a clause to the effect that the hospital or surgeon shall dispose of any tissues removed. In hospital cases, the hospital, rather than the surgeon, should have the right to dispose of removed tissues, in order that no controversies may arise between hospital and surgeon in complying with the requirement that all surgical specimens be sent to the laboratory for examination.

In the examination and disposal of stillborn infants, many hospitals proceed in a manner whose legality may be questioned. For the stillborn infant a certificate of stillbirth must be filed and a disposal permit must be issued. For the examination and disposal of a stillborn infant, permission is just as necessary as for the necropsy of a dead body.*

*Attention should be called to bulletin 83, by George H. Weinmann: A compendium of the statute law of coroners and medical examiners in the United States, Washington, D. C., National Research Council, 1931. pp. 240. This and bulletin 73, contain quotations from the written law and citations to court decisions that make them valuable sources of information when difficulties arise relative to the matters covered by their titles. When the physician encounters such difficulties, he should call the attention of his legal representative to these bulletins, since many attorneys-at-law do not appear to be familiar with the legal points involved.

SUMMARY

In the United States the application of medicine to law and justice, which may be said to constitute the medicolegal system of the country, occurs along several independent lines.

These are the investigation of certain classes of deaths, especially those which involve an element of criminality or negligence; the determination of the mental status and legal responsibility of those accused of crime; the determination of liability for personal injury or disease in accident and workmen's compensation cases; and the use of medical science in crime detection.

In the greater part of the United States the investigation of deaths, which is the aspect of the medicolegal system more fully discussed, is made by the office of coroner.

The obscure and political character of this office and the lack of clarity in the statutes defining its duties and authority combine to make the coroner's office an unsatisfactory agency in the administration of justice. There seems to be no very clear understanding, even upon the part of the medical and legal professions, of what the duties of such an agency should be and of what the needs of organized society are.

Of minor importance in rural communities, the office of coroner is a medicolegal and governmental agency that is called upon to perform a large volume of important public work in densely populated districts.

The coroner's office is not a necessary part of the American system of government, although the duties that it performs exist in every community. In a number of states the office does not exist, its duties being performed by the justice of the peace or the prosecuting attorney. In still another group of jurisdictions the coroner has been replaced by the medical examiner.

Reform of the coroner's office would require the transfer of the inquisitional duties of the office in criminal cases to the prosecuting, district, or state's attorney. In other cases the investigation to be made is medical in character. Abolition of the inquest would logically lead to abolition of the coroner's office and its replacement by the medical examiner system.

In a state medical examiner system, each densely populated county would require a single examiner's office. The less densely populated portions of the state should be subdivided into examiner districts, each comprising several counties. The size of the district should be governed by population, area, and transportation facilities.

In such a system there should be an agency maintained by the state, which might be known as a medicolegal institute. To such an agency medical examiners could submit materials requiring scientific investigation.

Certain problems relating to permission for necropsy, examination of stillbirths, and disposal of surgical specimens are discussed.

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THE MEDICOLEGAL NECROPSY

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This symposium by the American Society of Clinical Pathologists, a Society national in scope, upon the interesting and important subject of medicolegal investigation is of striking significance. I cannot conceive of a more fitting occasion to bring up such an important subject as the medicolegal necropsy. It is an opportune time to hold a symposium on these matters before a Society which is composed of trained and qualified clinical pathologists, familiar with laboratory work in its manifold aspects, and whose members have published valuable articles upon the clinical as well as pathological phases of medicine.

Some are probably not familiar with the post-mortem examinations of medicolegal cases; these have been handled by coroner's physicians, and in a few cities by a medical examiner. Specialism in medicine and surgery has grown enormously from necessity, and I do not hesitate to state that coroner's physicians, autopsy surgeons or medical examiners, to be thoroughly competent must be specialists, and by this term I mean that they should devote their entire time to this important sub-division of medicine.

That part of medicolegal medicine which concerns itself with the accurate determination of the cause of death, readily divides itself into two divisions. The first, and certainly for the medical profession, the most important division, is that which concerns itself with the cause of death, a strictly medical problem.

In the past this matter has been neglected. This neglect is traceable and involved in the evolution of medicine in our country. In the early days the physician served an apprenticeship with a physician or surgeon, and thus he slowly learned his medicine. Then there was a period of medical schools almost entirely pro-

prietary in character, followed by a period in which the splendid medical schools have been slowly evolved. Scientific research and all phases of medicine have made marvelous strides. But medicolegal medicine has not established itself anywhere in this country on a firm and progressive basis. The reasons for this neglect I may take up later in the discussion of the second division of my subject, namely, the legal or official status of the coroners and medical examiners systems of the country. Let me briefly state my conception of the qualifications of a medical examiner. The primary qualification is that he must be thoroughly versed in the practical side of pathology, hence, a long and varied autopsy experience. One of the most interesting of the problems encountered daily is that even after a thorough post-mortem examination we may be in doubt as to the cause of death. The pathologist who has had varied experience in hospital work of this character is, in my opinion, qualified to begin his task along medicolegal lines. He will, however, run across cases in which he will be puzzled, since his experience has not included just that kind of a case. To elucidate, when an unknown adult is found in coma, and there is no history connected with the death, and no previous medical history is available, frequently nothing is found which will indicate to an experienced man the cause of death. A thorough follow-up work is required. In a large city, most of these cases fall into the narcotic, the alcoholic, ethyl or methyl, and various poisons, over-doses from barbituric acid compounds, strychnin, and also very acute cases of sepsis.

After autopsy, the subsequent work entails chemic, microscopic and bacteriologic examination of the viscera and in homicidal cases examination of stains to determine if it is actually blood, and if blood, whether of human or animal origin as well as the examination for semen. Serological examinations may be necessary, involving the use of the precipitin reactions and also blood grouping tests which may lead to the identification of the person.

A little consideration will convince you that no one man is competent or able to perform alone what I have mentioned. However, the head of a department, the medical examiner of a county or of a city, should have enough experience to realize the

importance of the work at hand, sufficient knowledge to see that there is preserved enough tissue for chemical and microscopic examination. A prevalent belief is that all that is necessary is to retain the stomach content; an important test, of course, but alone it does not afford opportunity for making valuable observations and deductions which are necessary to elucidate a case of poisoning. Until absorption takes place from the stomach, the contents of the latter may be considered to be outside of the body. It is, therefore, of extreme importance to determine accurately and quantitatively, the amount of poison in the brain, liver, kidneys, et cetera.

The traumatic cases of a large city furnish the medical examiner with an enormous amount of material and the opportunity of making valuable observations and forming conclusions as to the circumstances of death.

Fat embolism plays an important rôle in the causation of shock following fractures of the large bones, and also it may be a cause of death. A valuable article by B. Morgan Vance¹ has called attention to the importance of this subject, and brought out the fact that while fat emboli frequently occur after trauma to fatty tissue, especially in the shafts of the long bones, that severe and fatal cases of embolism are exceptional, indicating that the protective mechanisms of the organisms work with sufficient efficiency to prevent the absorption of the fat into the blood stream in any considerable amount. He called attention to the fact that fractures of the bones of the extremities are most apt to produce fatal emboli but in his series of fifty-nine cases of fractures of the bones of the extremities, only three individuals died as a result of fat emboli. Finally, the author called attention to the difficulty in diagnosing the condition during life unless the possibility of fat embolism is kept in mind.

The post-mortem findings in casualty cases are essential, when it is borne in mind that death may follow casualties days or weeks after the injury. I know of no group of cases in which greater difficulties are encountered in forming a positive opinion and in arriving at a just conclusion as to whether the accident really had a determining action in the resulting death. It is unwise to

wear spectacles of prejudice and extreme care must be exercised that a preformed opinion is not entertained. The medical examiner must be unbiased in the opinion he gives to the grand jury, to the trial court, and to the compensation court.

Accident and compensation cases should be handled in a manner similar to that of the Central European countries, and Switzerland, where valuable dissertations and books upon traumatic injuries, the so-called Unfall Kunde, or Unfall Medizin have been published.

All vehicular accidents should be the subject of a careful inquiry. The statistics of the New York office show that in nearly 25 per cent of the deaths from such accidents which take place shortly after the injury are found to have been in persons drunk at the time of their death. No special emphasis need be placed upon the important judicial aspects of this finding. In the past, great injustice has been borne by perfectly innocent drivers who have run over drunken people crossing against the traffic lights of a busy city, or during the early hours of the morning.

Many vehicular accidents are due to accidental falls by persons suddenly taking sick with vertigo or who are partially blind, and a thorough examination is required. The French have called attention to other classes of traumatic accidents. Deaths from occupational disease requires a long review. Let it suffice to call to your attention lead poisoning, acute and chronic, mercury, benzine, and the brilliant work of Dr. Harrison S. Martland upon radium poisoning, which is unique and a classic.

What are the measures to be adopted to improve the administration of medicolegal procedure? The members of our Society are admirably suited to perform the preliminary work of improving the situation. The problems presented by a large city differ considerably from those of smaller cities, towns and counties. The organization of a large city where volume has to be taken care of is more difficult, and yet the problems presented in small cities or counties are similar, and careful autopsy work must be performed by competent pathologists and follow up work along the lines I have indicated in chemistry, pathology and bacteriology.

I suggested a number of years ago that in small counties a hospital pathologist and the health officer might assume the rôle of medicallegal experts. Although the suggestion is a workable one, there are immense difficulties since throughout the country the coroner's physicians are appointed largely through political influence. The politicians or our rulers have no appreciation of the work involved and many of them do not consider the work necessary. Any progress or change suggested will be considered the work of a reformer, who is despised because he upsets the status quo.* Let us contrast the system prevalent in this country with the Central European countries, Germany and Austria, where for decades a hereditary intelligence has prevailed and high standards have been enforced. The people recognize the necessity of a thorough investigation of crime and of disease. The investigators are state officers with University training and experience and are appointed by the University. Before appointment to an important post they have served many years as full time assistants of experience. The work is supervised closely by the court, as to the form in which their reports are written, and in smaller towns and cities where the reports are made by less well known experts, they are subject to revision by superior experts appointed by the court to serve. In cases where the report is insufficient for the court, or for a reviewer to come to a decision, an exhumation is made, and another necropsy is performed. There are well-known legal institutes in which interesting cases are worked up, published either in the general literature or in the separate publications of the institute of origin. A continuous progress in the science of medicine is established. The Medico-legal Institute of Vienna publishes the "Beitrage zur Gerichtliche Medizine," of which Professor Dr. Albin Haberda is the editor.

*Too frequently we let past conditions control our present thinking. To quote from John M. MacInnis: "One of the great menaces to real democracy is that political interest and activities are frequently left to selfish exploiters who look upon politics as an opportunity to advance their own interest and feather their own nests. The fault lies in the men who are not willing to subject their personal interests to the larger interests of society and to give the state the time and service that are essential to good government."

We have nothing comparable to these institutes. Recently, an Institute of Forensic Medicine was established in New York City with meager funds, a praiseworthy effort on the part of New York University, and the Bellevue Hospital Medical School to improve conditions. Much is required to establish an institute of this kind according to modern standards. An animal plant with trained keepers, assistants versed in serology and bacteriology, enough section cutters and laboratory men to perform the necessary work in pathology, as well as in chemistry. A plant of this character requires of its workers much time, and money is necessary. In a large city the medical examiner and his assistants should be full time men without any other interest. Under the existing circumstances, it is not possible to demand from medical examiners that they be full time men because of the low salary paid to them, and the dread hanging over them like the Sword of Damocles that they may at any time be removed, and thus placed in the position in the future of not being able to obtain their living by the practice of medicine.

In Europe the need for money and advancement along the social scale, so-called, is not as urgent a one as in our country. I think you will be able to read my thoughts, namely, that the great obstacles to progress lies in the lack of understanding and education of those in whose hands our fate lies.

In a discussion of medicolegal medicine, it is well to bear in mind that the functions of the coroner's physician, autopsy surgeon or medical examiner, is purely investigatory. There is no need of entering into further detail as to what the essentials of these officers are. The autopsy must be performed by thoroughly trained and competent microscopists in a laboratory capable of running through and sectioning tissues for examination. Also a competent chemist with assistants and suitable apparatus and a bacteriologist who is familiar with the details of his work as well as one familiar with blood examinations. Without this, there is no progress, and the conclusions to be drawn from an incomplete necropsy are almost wholly untrustworthy, and grotesque failures of justice have been recorded from these.

A medical examiner must have training in the examination of

scenes, of the body or bodies found there, the examination of the clothes of the subjects of homicidal assault, the presence or absence of powder grains, also a working knowledge of fire-arms, the distance at which a gun may have been held, and also if the clothes have more bullet holes than correspond to the number of entrances and exits from the body.

Chemical examination for chloral, chloroform and alcohol should be made in all homicide or suspicious deaths. A complete record of the scene and of the autopsy and the subsequent work is essential for use in a trial.

The profession represented by the medical examiner must be authorized to determine the cause of death of any cases under his jurisdiction by necropsy when he deems it necessary. Lawyers and coroners are not qualified to determine this question as it is a strictly medical one. Fortunately, in New York, we have complete authority and we do not have to ask permission from any one how we should handle any given case. In some states the District Attorney is the one to be appealed to for permission. A ridiculous situation, since it is a strictly medical problem. Many jurists believe that the cause of death is objective, that an autopsy should cease, for instance, when the cause of death has been supposedly reached, seeming to hold the view that the cause of death is an object, to be removed from the body when seen.

What is meant by the cause of death? Too little attention has been given to the scientific aspects of this problem. For instance, how commonly we hear that a man has died from a fracture of the skull. A fracture of the skull does not cause death in itself. Often the inaccuracy of statement by an expert may lead to serious injustice. The teaching of applied and experimental physiology should always be invoked. The rapidity of death following injury—how far can a man walk or run after being shot—these determinations are not always readily ascertainable. But a little knowledge of physiology may serve to elucidate the case for the judge and jury. I do not wish to enter into any discussion of the relative merits, and demerits of the coroner against the medical examiner system. It seems to have been overlooked that the masters of forensic medicine, Taylor and others in England, have

for the last eighty years been decrying the archaic system of the coroners in England.

Truth compels the conclusion that glaring deficiencies exist in the administrations of the coroner's as well as the medical examiner's system of the country. The remedies are known, but not easy of application, for the treatment is not entirely in our hands. Experienced pathologists in control with well equipped laboratories and sufficient clerical forces, stenographers and photographers are indispensable.

The second division of the talk may be made very briefly. First, educate the public and our legislatures to the importance and extent of the subject of medicolegal investigation.

Second, teach the value of the necropsy. A well planned campaign to overcome the prejudice of the public to examination of the dead is necessary. The medical profession is somewhat responsible for the antagonistic attitude of the public. The contrast between our country and the central European countries is noticeable in this regard. To illustrate, all the professors who taught me during my post-graduate studies in Vienna were thoroughly examined after death. There is very little racial prejudice to post-mortem examination except among the orthodox Hebrews.

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PERFORMING THE MEDICOLEGAL NECROPSY

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The medicolegal necropsy, in accordance with the laws of most states, embraces those cases of death which may have been induced by homicide, suicide or accident. Due to the latter, it must at once become apparent that a great many medicolegal necropsies come within the purview of the compensation laws of the various states, opening and embracing the relatively new field of occupational diseases and injuries.

For purposes of simplification, medicolegal necropsies may be classified into the following six major groups:

- (1) Crimes of violence (shooting, strangulation, stabbing, infanticide, et cetera)
- (2) Sex crimes (rape, et cetera)
- (3) Poisonings (including homicidal, accidental, occupational)
- (4) Traumatic accidents (automobile injuries, falls, et cetera)
- (5) Still-births, malformations, birth injuries
- (6) Sudden (accidental) deaths

Modern criminal society, as depicted by the gunman, racketeer and other perverts of humanity, leans much more to the quick violent method of death, that is, shooting, stabbing, strangulation, et cetera, than to the ancient "fine art" of the poisoner's hand, an art they have relegated to the limbo of the past.

The medicolegal necropsy, while demanding astute knowledge and care on the part of the pathologist in establishing the cause of death, requires much more than a painstaking necropsy. A well trained medicolegal pathologist can be of inestimable service to the police officers at the scene of death. The position of the body, the instruments causing death, the condition of the surroundings, the presence of a box or of a bottle, the odors in a room—all are of importance in arriving at a conclusion as to the cause of the individual's death.

When the pathologist is ready to proceed with the examination of the remains, having first satisfied himself as to the identification of the body, either by a relative, friend or the police officer, he should begin with a minute inspection of the external body, dictating to a competent stenographer as he proceeds with his findings, both negative and positive. It is absolutely necessary to have an identification of the body, and dictation of the findings must be made as the necropsy proceeds in order to be competent evidence, although, of course, correction of a draft of a protocol is permissible. During the inspection, the examiner must always bear in mind whether a lesion is antemortem or post-mortem and whether it may or may not have been self-inflicted. All bodies must be measured as well as weighed. For the latter an ordinary platform scale is suitable. All the organs must also be weighed and for this purpose an ordinary dial scale or the usual grocer's balance is satisfactory.

The external inspection of the body is probably of greater importance in the majority of medicolegal cases than examination of the remainder of the body, since most criminal deaths at the present time are crimes of violence which leave external evidences, rather than the more subtle methods of poisoning. This subject is, therefore, dealt with in some detail. It is well to conduct the inspection in an orderly manner, commencing with the hair on the scalp. The condition in which the hair is found should be noted—its texture, color (dyed?), blood or other stains or admixtures, et cetera. The condition of the scalp should also be noted, especially for wounds, hemorrhage, et cetera. The eyes should be examined with particular regard to the state of contraction of the pupils and the opacity of the cornea and lens. The ears, nose and mouth should be examined for discharges (blood, pus, et cetera). The condition of the teeth, tongue, and other mouth parts should be noted. Rigor mortis, discoloration of or marks on the skin, surface temperature, should be noted. Puncture marks or old needle scars, especially in the arms and thighs, may be of value in directing the examiner's attention either to a case of poisoning or drug addiction.

Careful inspection of the entire body should be made for ec-

chymoses, injuries, wounds, scars, fractures, and anomalies. Very often a bullet wound made by a 22 calibre gun may be overlooked. In the case of bullet wounds, the condition of the surrounding skin should be searched with a magnifying lens for powder marks, and burns. Search for the wound of exit must be carefully made. Knife wounds or lacerated wounds of any kind should be carefully examined as to their nature, size and direction, since the latter particularly may yield a clue to injury to an internal viscus, the position of the assailant, and the appearance of the wound may give an indication of the type of instrument used. Often a syphilitic scar on the penis or extremities may be a clue to a natural death which had been thought at first to be of a suspicious nature. Ecchymoses may often aid in interpreting the position in which the individual dropped at the time of accident or injury. Ecchymotic areas about the neck with edema of the tissues, tongue, and other parts of the head and neck, and imprints of fingers may indicate strangulation. Rigor mortis, the temperature of the body, and the clearness of the lens are, within reason, relative points as to the time of death of the deceased. The determination of temperature is a very variable factor, depending upon the temperature of the place in which the individual was found, whether he had a fever just prior to death, or whether other conditions pertain. In general, however, it may be said that the temperature drops one degree for every hour post mortem until the surrounding temperature is reached. The opacity of the lens and cornea is likewise a variable factor, depending on the age of the individual, his state of health, the condition of his eyes, and other general and specific conditions, but here again it may be taken as a rough index as to when death occurred. In general, whenever a haziness is found in the cornea, it may be said that death ensued at least twenty-four hours previously.

It is advisable to finger print the individual, not merely as an aid to the identification of the deceased, but to compare the prints with others obtained at the scene of the crime (or accident) or with those attained on previous occasions. Finger printing has been adopted as a routine procedure in most of the European.

countries, since it is used there as a method of identifying anyone. It is to be hoped that an enlightened people will insist that similar laws be adopted in the various states of this country.

Frequently anomalies (supernumerary fingers, toes, et cetera) or peculiarities of the individual may be found, especially in the upper extremities. Thus, an exostosis of the index or middle finger may be the result of the position in which the individual held his pen or pencil and this may be an aid to identification.

Examination of the teeth and even dental impressions are of value, especially for purposes of identification, and a chart such as is used by every dentist should be included in the records of the case, especially if the deceased is unknown.

Although most postmortem rooms are not equipped with x-ray apparatus, it nevertheless is often desirable to take complete pictures of the corpse, and facilities for x-rays and photography should be provided. This is of especial value at times in locating a bullet, in revealing a fracture, and for other purposes.

The question of sudden death from natural causes is one which the medicolegal expert will constantly encounter, and it is well to bear it in mind. Of paramount importance is the so-called status thymico-lymphaticus death. This condition has frequently been the subject of considerable dispute. Only recently a prominent German pathologist questioned the relative importance of status lymphaticus as a cause of death. He based his opinion on post-mortem observations made during the late war, in which he claims that no cases of "status" death, or one in which status lymphaticus played a rôle in the cause of death, could be found. However, every experienced pathologist, we are sure, has encountered bodies showing the characteristic anatomic landmarks of status lymphaticus and presenting ruptured hypoplastic vessels, and other conditions associated with this condition.

In addition, one must bear in mind the cases of sudden death without any recognizable lesion. Among these are included true angina pectoris, deaths from shock or syncope, anaphylactic death, diabetic coma, insulin shock, and acute ethylism. Of these types, some may at times be difficult or even impossible of recognition or explanation at the autopsy table. We refer especially

to anaphylactic deaths from foreign protein sensitization where the foreign protein is of such minute amount that it is impossible of detection by our present chemical methods, or, as with excessive doses of insulin, the injected substance is quickly metabolized.

A miliary embolism, edema of the glottis, or a foreign body in the larynx or trachea, are lesions causing sudden death that are frequently overlooked.

Finally, it may be said that sudden death in the young is generally caused either by a disturbance of the nervous, respiratory or intestinal systems or by status lymphaticus. On the other hand, in adults, lesions of the circulatory system are of the greatest importance.

It is not the object of this paper to discuss the various methods of conducting an examination of the interior of the body, since this must be dependent upon each individual's tastes and training and the purpose for which the examination is made. The incisions employed in opening the body cavities may be varied. In the usual procedure, an incision is made commencing either at the suprasternal notch or under the lower chin and terminating at the symphysis pubis. Latterly, in attempting to conform with modern dress, we have, especially in females and sailors, made a V-shaped or elliptical incision, commencing under the breasts and running to each shoulder joint. This is dissected upward and forms a complete flap which, when sutured, is not visible after the body is properly clothed. This incision connects with a median incision just below the breasts and extends to the symphysis pubis.

After the body is opened and note made of the panniculus adiposus and the condition of the muscles, the peritoneal cavity is examined. The position of the omentum and of the abdominal organs in situ is noted. Examination is made for fluid, gastrointestinal contents, blood, tumors, inflammatory exudates, tubercles, et cetera. The pelvis is inspected and the height of the diaphragm noted. Frequently a ruptured or perforated viscus causes blood, bile, stomach or intestinal contents to be found in the abdominal cavity. Bullets, gall-stones or enteroliths may be

found in the abdominal cavity. Lesions due to fat necrosis from an acute hemorrhagic pancreatitis may be scattered throughout the cavity. Pus beneath the liver may be found secondary to a comparatively mild inflammation of the appendix. A gangrenous omentum or piece of strangulated intestine or a mesenteric thrombosis may be encountered. Bleeding from a small wound in the liver or spleen may result from a perforation by a rib caused by compression or a fall. Frequently such abdominal viscera as the liver, spleen, stomach, kidney, or bladder, are ruptured by an automobile or other heavy vehicle passing over the abdomen. Ruptured viscera, particularly the stomach, have been found in infants and young children following a simple convulsion. A ruptured spleen and fatal hemorrhage from a fulminating malaria or leukemia is not unknown.

It is not our intention in this paper to discuss rape, criminal abortion, postpartum sepsis, and other criminal deaths in detail, since these constitute one of the most difficult and controversial chapters in the entire history of medicine and have been the object of discussion and argument since the dawn of civilization. However, several cardinal points must be kept in mind. In the question of rape, the presence or absence of a hymen or a lacerated hymen must be sought, and, in young children, a lacerated perineum is, as a rule, mute evidence of rape. Smears from the cervical os or from the vagina may show the presence of spermatozoa, but care must be taken not to mistake parasites (*Trichomonas vaginalis*) for the former. In cases of criminal abortion, the impress of a tenaculum in the cervix, together with recent lacerations of the cervix, may be considered as presumptive evidence of abortion. The presence of a corpus luteum together with the gross and microscopic finding in the uterine wall of the placental tissue reaction is conclusive evidence. In this connection, it is desirable to call attention to a case which illustrates the importance of routine toxicological examinations in legal medicine.

A body came to necropsy in which abortion was suspected, the patient having died in the abortionist's office. The latter stated that the patient had come for an examination and died in his

examining room. At necropsy, the uterine contents were almost intact; there were, however, tenaculum marks on the cervix and sufficient separation of the placenta to cause fatal hemorrhage. The remainder of the postmortem examination was negative. Chemical analysis of the viscera revealed very large amounts of chloroform, and particularly in the brain. The abortionist was convicted and sentenced for manslaughter on the evidence of criminal interference with pregnancy, the presence of chloroform and fatal hemorrhage.

Continuing the examination, the next step may be the removal of the intestines and any or all of the abdominal organs for preservation as evidence, or for further study. Frequently cultures, smears, chemical and microscopic examination of the contents of the cavity, and particularly of the stomach, intestine, liver and kidneys, must also be made.

The sternum and costal cartilages are next removed and an orderly inspection of the thorax and its contents is made. After the thymus is inspected and removed, it is perhaps best to examine carefully the pericardium and its contents, since a stab or small calibre bullet wound frequently leaves very little evidence in the pericardial wall. On the other hand, the slightest injury to the heart or great vessels causes a hemopericardium. Before the heart is removed, its anterior surface should be inspected for injuries. Then the pulmonary artery should be incised in situ so as not to overlook a pulmonary embolus. Following this, both thoracic cavities should be examined for blood, pus, fluid or other foreign material. The heart is examined next on its posterior surface, after which it is removed, opened and examined. If an aortic aneurysm is present, it may be well to remove the lungs so that the heart and aorta may be removed en masse. In this connection, a coronary thrombosis or a ruptured aneurysm or ruptured aorta may be of the utmost importance. The state of the blood, whether it has undergone postmortem coagulation or is more or less liquid, should be noted, since, in cases of poisoning (alcoholism, gas, and under certain other conditions), it is, as a rule, more or less liquid. The lungs are next removed and in *new-born* or *still-born* children should be immersed in a basin of

water to determine if air had ever entered them. The lung, all its bronchi and the vessels should be examined. Small bronchial tumors, water or other fluids, foreign bodies in the bronchi, abscesses or pulmonary emboli are easily overlooked. The mediastinum must be examined, particularly for tumors, dermoid and echinococcus cysts.

The larynx and trachea must be removed and inspected. Frequently sudden death in an alcoholic individual may be explained by a particle of food obstructing the larynx and not by any excess of alcohol. In the case of a child found dead in bed, necropsy disclosed the presence of a lead pencil which completely obstructed the larynx and caused edema of the glottis. In this connection, it is important to call attention to the possibility, especially in children, of a diphtheritic membrane obstructing the larynx. Edema of the glottis following trivial injury to the neck or mouth must not be overlooked.

The esophagus must be split open and examined. Pin-point perforations may explain a suppurative mediastinitis or pleuritis, and larger openings may account for food contents in the thoracic cavity. Ruptured esophageal varices likewise may explain a fatal hematemesis. The thoracic duct must also be examined for perforations from bullets, stab-wounds, and other wounds, and rupture as a result of trauma or disease; particularly tuberculosis, must not be overlooked. The organs of the neck—lymph nodes, tongue, thyroid, et cetera—must also be inspected. Of great medicolegal importance is a fracture of the hyoid bone and thyroid cartilage leading to suffocation.

The skull requires most painstaking observation. Wounds and ecchymoses, of the scalp are of importance. The measurement of the cranial diameters and general shape and contour of the skull should be noted. The scalp as a rule is reflected by incising it, commencing behind one ear and bringing the incision across the cranial vault to end behind the other ear. The flaps are then reflected anteriorly and posteriorly. The cranial surface is next examined for hemorrhagic areas, fractures and other injuries. The skull is then opened by a circular incision through the occipital protuberance and about midway through the frontal

bone. After removal of the skull cap, the external dura is inspected, especially for pachymeningitis and for traumatic injuries. The dura mater is next removed and its inner surface inspected for similar lesions. The surface of the brain is then examined and the organ is carefully removed in its entirety, cutting off the cord as low down as possible. The vessels at the base of the brain are then examined, especially for aneurysms, thrombi or emboli. Next the brain is opened by incising the lateral ventricles. The quantity of fluid, the condition of the choroid plexus, the ependymal surface, and the presence or absence of blood is noted. All the ventricles are opened and their surfaces inspected. The condition of the pineal gland should be noted. Following this, the brain is cross-sectioned and any change in the consistency of the brain, its vessels or covering noted. The cerebellum is likewise sectioned and the medulla opened by multiple incisions. The dura covering the base of the skull must then be examined and removed. Following this, the floor of the skull must be inspected for fractures and penetrations. The foramina and their contents should also be examined. Next the middle ears are opened by perforating the petrous portion of the temporal bone, and careful examination is made. Similarly the pituitary gland is removed and entry is obtained into the sphenoidal, frontal and ethmoidal sinuses. The orbital cavity may be opened and examined and the eyes also, if it is desired, may be removed in this way.

The spinal cord and, for that matter, the brain may be removed before the rest of the body is opened, but as a general rule this procedure is left until last. The cord is removed by a long longitudinal incision over the spinal column and the latter is usually opened by sawing through the lateral spinal processes. The spinal dura is next inspected and the nerves running from the cord should be examined. The cord is then removed, as a rule, in its entirety. In general, it is safe to say that the cord is usually relatively free from injuries except that now and then a bullet may penetrate its massive encasement, or a violent accident may cause a fracture of one or more of the vertebrae followed by compression myelitis. The cause of rapid, otherwise unexplain-

able deaths, especially in children, may be found in the cord, in the form of a fulminating anterior poliomyelitis, though this may generally be observed in the medulla oblongata.

It may be necessary to incise and examine the extremities when there have been fractures or in cases in which osteomyelitis, tetanus, gas-bacillus infection or a cryptogenetic sepsis has induced a fatal outcome from an otherwise trivial injury.

In conclusion, while only a brief and sketchy outline of the average medicolegal necropsy has been given, it is hoped that sufficient interest has been aroused in those pathologists who are called upon to perform medicolegal work, that they will seek further in this most important and difficult field of pathology. It must be recognized that a tremendous responsibility, namely, the life or death of an accused, at times rests solely and squarely upon the shoulders of the medicolegal expert and in the result of his findings and the opinion that he renders. The medicolegal pathologist can perfect himself in this work only by continued experience, careful analysis of his findings, and constant study of the literature, which is vast, indeed, and increasingly so, though, unfortunately for many, chiefly in foreign languages.

The importance of careful and painstaking examinations and analyses carried out with an open mind, not swayed by emotional outburst of press or public, or clouded by preformed conceptions gathered from similar cases, must be stressed. The medicolegal expert must be well trained in all fields of general pathology, including serology, and bacteriology, but especially in pathological anatomy, so that he can recognize a non-medicolegal death. In addition, he must have obtained theoretical as well as practical knowledge in medicolegal work. Such knowledge and experience are difficult to obtain at present in this country, since few schools give even the barest outline of what constitutes a medicolegal case. It is hoped, however, that an awakening conscience on the part of physicians will lead to greater attention to this difficult branch.

PATHOLOGICAL ANATOMY OF DEATH BY DROWNING

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To correctly interpret the lesions found in the body of a drowned individual, one must be well versed in the mechanism of death, clearly visualize the rapidly ensuing phases of struggle and defense of the submerged victim against overpowering forces, and have in mind the possible pre-existing pathological affections of the internal organs, particularly of the lungs and heart.

The post mortem examination represents only one phase of the investigation, where a question is raised as to whether or not an accidental, suicidal, or homicidal drowning occurred. Incidentally accompanying circumstances and many other conditions and situations must be thoroughly investigated and considered and then carefully correlated and evaluated.

My discussion is limited to the pathologic anatomy and to the differential diagnosis at the necropsy table.

Death by drowning, if uncomplicated, is a genuine asphyxial death, with occurrence of phenomena so commonly observed in this type of unnatural death.

APPEARANCE OF THE RESPIRATORY ORGANS

When the thoracic cavity is opened routinely, the lungs of a drowned individual do not, as they do under ordinary situations, recede or partially collapse, due to atmospheric pressure; but, on the contrary, they appear greatly distended, inflated, and their anterior margins either reach or even trespass the mediastinal midline, overlapping one another. The voluminous lungs completely fill up the space allotted them in the bony cavity of the chest, pressing against the ribs and pushing the intercostal spaces outwardly and the mediastinal pleura medially. The front bor-

ders of the lobes are not thin and sharp, but are well-rounded, and the basal edges exhibit this same appearance.

In observing the pleural aspect of the lungs, one does not meet with an even and smooth outer surface, but notices slightly projecting, prominent areas of a lighter color than the rest of the lung, often decidedly lobular in distribution, resembling an acute compensatory emphysema. Markedly distended air vesicles are readily discerned through the pulmonary pleura on the external aspects of the lobes.

The imprisoned, compressed air may be forced, through microscopic tears of the alveolar walls, into the interstitial frame of the lung, and then small air bubbles appear in a rosary-like arrangement beneath the pleural membrane.

Irregularly scattered patches of a suggested hemolytic imbibition are not uncommonly seen through the pleural covering, but pleural ecchymoses are rarely encountered.

The spasmodic, deep inspirations and the obstructed and insufficient exhalations finally lead to a marked or extreme ballooning of the lungs, the extent thereof depending upon the duration of the respiratory efforts. The volume of the retained air in the lung may exceed, by three to four liters, the amount present in an average lung. The degree of emphysema resembles in a certain way that found in fulminant types of acute diffuse bronchiolitis. Asphyxiation quickly sets in. One may ponder upon whether carbon dioxide saturation of blood exerts an ominous influence upon the vital centers of the medulla oblongata, contributing in causation of death.

In different individuals, the degree of sensibility and reflex reactions of the pharyngolaryngeal mucosa vary considerably and play an important rôle when any kind of foreign material encroaches upon these structures. The defense mechanism is accordingly influenced, namely, it is lowered or increased. If the first is true, water will rush into the respiratory organs, shortening the fatal period of struggle, while the individual possessing prompt reflectory responses will survive a longer time, in spite of the fact that he is caught defenseless in the drowning medium.

PULMONARY EDEMA COMPARED WITH WATERY LUNG

The lungs are not quite as heavy or as doughy throughout as they are in an edema resulting from other conditions, as seen, for instance, in cases of gradually failing function of the left side of the heart (transudation due to passive congestion). The consistence of the pulmonary tissue is not uniform, the dependent parts possessing a more pronounced doughy-elastic character. When one presses the lung with the tip of the finger, a circumscribed, sluggishly-disappearing depression results, similarly to that in edema of the ankle.

The cut surfaces of the lungs exhibit changes which can be differentiated without difficulty from other types of edema, such as cardiac, inflammatory, toxic, or certain asphyctic edemas.

While the volume of a normal lung is reduced when the organ is sliced with a knife, because of escape of air, and the parenchyma sinks below the level of the incised pulmonary pleura, the cut surfaces of the lung of a drowned individual do not retract.

The fluid quantity is considerably less than in a transudative or congestive type of pulmonary edema, only a small or moderate amount of frothy, clear fluid exuding. The same observation is also made when one compresses the incised lobe laterally and notes the comparatively small amount of expressed fluid oozing upon the cut surface. The drowning fluid forcefully and rapidly penetrates into all the structures of the lung and cannot be liberated easily.

The pulmonary parenchyma is more or less pale, except for hypostasis found in the dependent parts, and is not cyanotic and edematous as, for example, in cardiac congestion and edema, the moisture being comparatively far less pronounced, as mentioned before. The degree or intensity of the cadaveric hypostasis depends, of course, upon how soon after death the body was removed from the water and examined.

Prominent areas, identical in appearance to the projecting, patchy areas of emphysema visible on the pleural aspect, are also evident on the sliced surfaces. The air caught within the air vesicles during the dyspnoic stages distends the alveoli to a high

degree and does not always and in all places allow the invading fluid to enter them.

BRONCHIAL MUCUS HYPERSECRETION

The penetrating fluid acts as an irritative agent upon the entire mucous lining of the bronchial tree, causing congestion, and a hypersecretion of mucus, intense in character, ensues simultaneously. It is because of this fact that slimy material is so prevalent in all the respiratory tubes and that mucous shreds are found far down, within even the smallest bronchial branches. Small threads or minute clumps of mucous matter are noted quite regularly, but in varying quantities, in the liberated fluid obtained from the cut lung tissue.

Drowning fluid may or may not be present in the upper respiratory tract, depending upon the position of the body, handling during and after removal, artificial respiration, et cetera. If present, it contains finely distributed, minute air bubbles and slime. A clear froth, oftentimes abundant in amount, is seen at the nasal openings and mouth, and either partially or totally fills up the pharyngolaryngeal space, the trachea, and the main bronchi.

FOREIGN BODIES IN DROWNING FLUID

Should the drowning medium be turbid, colored in some way, and contain tiny or some barely distinguishable particles of vegetable or animal matter or origin, the fluid exuding from the lungs will be similar in appearance and the larger foreign bodies contained therein may be detected even by the naked eye. Frequently, such particles are seen sticking loosely to the mucous surface of the trachea and bronchial ramifications.

Microscopical analyses of the fluid scraped from the cut surfaces of the lung and of the medium in which the body was found should never be omitted, since in cases in which the autopsy does not yield positive or conclusive findings, such comparative microscopic examinations must be resorted to as the main means of establishing a correct diagnosis. But one should thoughtfully consider the fact that only presence of such particles in the

bronchioli and within the air sacs, occasionally within the interstitial tissue, is conclusive of their having been aspirated during life, since foreign bodies may arrive in the bronchial branches, even in the small ones, after death, especially if the body was immersed in deep water or in fluid medium under pressure, or if artificial respiration was applied.

ATYPICAL CASES

The findings mentioned above are met with in ordinary or typical cases of drowning, when examined soon after death and in which the lungs were not affected with chronic or acute conditions of any kind before. Pre-existing pathological changes may considerably change the entire picture.

This is particularly true in instances of firm fixation of the lungs to the thoracic wall as a result of an old, healed pleurisy. If the lung presents adhesions over only a circumscribed area, one may notice that the pulmonary tissue corresponding to that area does not show changes as described; the contrast between the findings in this adherent part and in the non-adherent portions of the lungs is clearly obvious, upon comparison. Such differences in gross appearance are even more striking in cases of total unilateral adhesion of the lung, since the fixed lung does not show findings of such a typical nature as does the freely movable, healthy lung. A comparison of the two illustrates this point very distinctly.

A pre-existing chronic pulmonary emphysema may confuse one in interpreting the significance of that condition. A critical weighing of this with the other enumerated points will aid one in differentiating it from a recent ballooning of the lung due to the effect of drowning.

Putrefaction may completely alter all the signs significant of death by drowning, and in advanced stages the diagnosis is rendered quite impossible. The thoracic cavities then contain a considerable quantity of a dark hemolytic fluid which had transuded from the lungs, and the costal pleura becomes hemolytically discolored throughout. The lungs appear small, collapsed, and present well-developed cadaveric changes. The frothy condition in the

air passages, a sign so characteristic of drowning, completely disappears. Cadaveric hemolytic processes discolor all the tissues.

POST MORTEM ENTRANCE OF FLUID

A post mortem penetration of fluid into the lungs may cause them to become heavy and quite moist, thus simulating to a certain extent the anatomical picture in cases of actual drowning. But, the emphysematous component, the ballooning of the lungs, and the fine frothy contents in the bronchial tubes are absolutely missing. Every single detail must be thoughtfully noted and cautiously reckoned with in order to avoid an erroneous interpretation and conclusion.

MUSCULAR HEMORRHAGES

A particular condition, which may have a certain criminalistic significance, is presence of hemorrhages in the soft tissues of the neck, within or between the muscles of the neck, for instance, involving the sterno-cleido-mastoid, sterno-hyoid, sterno-thyroid, et cetera. Occasionally, such hemorrhagic infiltrations may develop within the pectoral muscle or in the muscles at the nape (trapezius). These hemorrhages are not the result of any external violence but occur on account of minute tears of muscle fibers during the dyspnoic and asphyctic stages, which cause spasmodic muscular contractions (tonic and clonic convulsions), especially flexion of the head. Plainly visible lacerations of such muscular groups are seen in frozen bodies and are often artificial effects from handling the dead body.

If such muscle hemorrhages are detected, a strangulation should not be summarily assumed, or rejected. A close inspection of the skin of the neck for presence of marks of constriction is imperative. A dissection of the neck organs should follow, in order to ascertain whether injuries to the laryngeal structures or to the hyoid bone occurred. Evaluating the results of these examinations and considering the location of the muscular hemorrhages found, particularly if they are developed distantly from the laryngeal region, will safeguard one in deciding whether ante mortem attempts at strangulation were made.

SKIN PHENOMENA

Usually the entire surface of the body appears very pale and anemic because of contraction of all the peripheral blood vessels. Shock-like phenomena may occur, particularly in individuals susceptible to effects of cold water (urticaria-like reactions in the clinical picture). The blood is rapidly forced into the abdominal organs, flooding the splanchnic territory. This might explain certain instances of rapid, apparently sudden death in persons unexpectedly submerged, on account of anemia of the brain and inhibitory reflex actions. At autopsy, one may, accordingly, find indications of such an event.

It is a general belief that "goose-flesh" (*cutis anserina*) is commonly found in cases of drowning; however, it has no diagnostic value. It is indisputed that cold water will produce it during life. But, "goose-skin" may develop after death as a result of rigor mortis of the *arrectores pilorum* muscles. I have often caused it mechanically by stroking the skin of the body after death with the tip of my finger or with an instrument, and have produced it up to eight hours after death, which phenomenon gradually subsides together with the disappearance of the mechanical contractility of the skeletal muscles.

The skin of the palmar and plantar surfaces shows, after several hours of immersion, a bleached and coarsely wrinkled appearance, proving only that these parts of the body were in water for some time, either before or after death.

If wounds or bruises are detected, their ante mortem or post mortem character must be established and their origin, in correlation with circumstances under which the body was found, logically explained.

OBSERVATIONS OF OTHER INTERNAL ORGANS

The heart is flabby, as a rule, and its right side is dilated and filled with a dark-red fluid blood. As in some other types of asphyxial death, the blood does not clot. The drowning fluid, which is rapidly absorbed into the system, leads to hemolysis of the blood. This disintegration of the blood can be readily ascertained by examining the heart cavities, and then it is more

pronounced in the left than in the right ventricle. The cadaveric hemolysis, on the contrary, is more accentuated on the right side of the heart and within the field of the portal vein.

The spleen exhibits, as is generally true of cases of asphyxiation, a slight decrease in size, as indicated by a wrinkling of its capsule and a gray-red, somewhat anemic appearance of its pulp. On the sliced surfaces of this organ, the blood does not exude as richly as it does upon incision of the liver.

The submerged victim, attempting to breathe in his desperate struggle for life, swallows some water, and his stomach, in addition to its varying amounts of fluid already present, will then contain a considerable quantity of liquid. In adults, the ingested fluid may even trespass the pyloric barrier. The swallowed fluid may have, grossly and microscopically, the same characteristics as the medium in which the individual drowned. But this finding can not be used as a conclusive sign of death from drowning, since water may enter the stomach after death, similarly as it may the upper respiratory tubes.

Complete obstruction of the nose and mouth by fluid or water is sufficient to cause an individual to drown; a total submersion of the body is, naturally, not necessary. I have investigated cases of persons who in deep alcoholic intoxication fell face down, vomited, and asphyxiated by, or drowned in, their own vomitus. The lungs presented an extreme degree of ballooning; the aspirated food particles acted in a valve-like fashion, permitting entrance of air but hindering its exit.

While it is obvious that in a paper of this length not all the details of the lesions and conditions produced by drowning could be discussed, nevertheless the most fundamental and interesting lesions have been indicated and their significance exposed.

TOXICOLOGY IN THE MEDICOLEGAL NECROPSY

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Toxicology is the science of poisons. It deals with (1) the properties of poisons, (2) methods of detection and quantitative determination of the poisons in body tissues and fluids, (3) the lethal or fatal doses of various poisons, (4) the action of poisons on living tissue and (5) the proper antidotes to be used to counteract poisoning.

There is no substance known that acts in a harmful manner under all circumstances and in all doses. One cannot set a definite fatal dose for any one substance because it varies according to (1) the form in which it was taken (degree of solubility), (2) whether food was in the stomach at the time of ingestion (3) through what channels the poison was taken (gastro-intestinal tract, inhalation, absorption through the skin, by hypodermic, et cetera), (4) the quantity of poison actually absorbed into the internal organs, (that remaining in the gastro-intestinal tract has no bearing on the poisoning other than the degree of local irritation), (5) individual differences of tolerance and idiosyncrasies,* (6) age, sex, physical condition and habits of the individual and (7) rate of detoxication and elimination of the poison from the body.

One cannot distinguish between a medicine and a poison without knowing the amount taken (dosage). For example, $\frac{1}{32}$ grain of strychnine is a tonic, while $\frac{1}{2}$ grain is a violent poison to the average adult. Table salt, sugar, and even water, if given in great excess, may act as poison. Extremely small amounts of a substance may be entirely inert physiologically, but increasing amounts of the same material may be therapeutic, then toxic, and finally fatal.

* If a drug is given properly, in good faith and in accepted medical dosage any unfortunate results would not be considered as criminal poisoning.

DEFINITION OF POISON

A poison is a material of mineral, plant, or animal origin which, if introduced into the living body or brought into contact with any part thereof, in a soluble form and in sufficient quantity (with the exception of glass, needles, et cetera) will produce ill health or death.

The fatal dose cannot be definitely and accurately stated. It is the average amount of poison taken per kilogram of body weight that proves fatal. Due to individual differences, however, some animals may recover from this amount of poison while others may die from less than this amount. In man the lethal dose is taken as the smallest amount known to have proved fatal. This value for the lethal dose, however, is far from satisfactory for the following reasons:

(1) The history may be misleading or even erroneous; how much, if any, was lost by vomiting or excretion is not accurately known.

(2) The quantity absorbed and in the circulation and in the tissues is of importance only; that part of the material that is left in the gastro-intestinal tract after death has no bearing on the toxicity and death, with the exception of local corrosive action, if any.

(3) The condition of the individual, such as age, state of health, idiosyncrasies, et cetera, plays a part.

(4) The fatal dose differs when given in one or two large doses (acute poisoning) as compared with a small amount given over a prolonged period of time (chronic poisoning).

At present the "lethal dose" is still a hazy term and should not be given too much importance in the courts. In order to arrive at a better understanding of what constitutes a fatal dose in a case of poisoning, one must strive to get all the information possible regarding the previous condition and habits of the deceased, and must quantitatively analyze the various organs, tissues and body fluids (except the poison left in the gastro-intestinal tract). From the analyses, one must estimate as closely as possible how much of the poison had been actually absorbed into the body proper. It is the quantity absorbed and not the quantity taken

that is of importance. The smallest amount of poison actually absorbed into the body and positively known to have proved fatal to an average healthy person, should be known as the lethal absorbed dose.

LEADS AND CLUES

From a toxicological standpoint the first things that should command one's attention in a medicolegal necropsy are the leads and clues. Since analyzing for hundreds of poisons necessitates much autopsy material and is very time-consuming, a clue or lead toward some poison or group of poisons is very helpful. To get these leads there are two sources of information.

(1) The history and symptoms.

(a) *Vomiting, purging, abdominal pains* may indicate poisoning by arsenic, antimony, aconite, acids, alkalies, barium, cantharides, copper, digitalis, iodine, mercury, phosphorous, phenols, wood alcohol, veratrum, zinc.

(b) *Convulsions* may indicate brucine, camphor, cyanides, santonin, strychnine.

(c) *Coma* may indicate alcohol, atropine, carbon dioxide, carbon monoxide, chloral, chloroform, cyanides, ether, paraldehyde, phenols, opium group, sulphonal, veronal group.

(d) *Dilatation of pupils* may indicate belladonna, cocaine, gelsemium, hyoscyamine, nicotine, stramonium.

(e) *Contraction of pupils* may indicate muscarine, opium group, physostigmine, pilocarpine.

(f) *Paralysis* may indicate cyanide, carbon monoxide, carbon dioxide.

(g) *Slow respiration* may indicate carbon monoxide, opium group.

(h) *Rapid respiration* may indicate atropine, cocaine, carbon dioxide.

(i) *Delirium* may indicate atropine or cocaine.

(j) *Dyspnea* may indicate carbon monoxide, cyanide, strychnine.

(k) *Cyanosis* may indicate acetanilide, aniline, benzene, opium.

(1) *Pinkish discoloration* may indicate carbon monoxide or cyanide.

(2) Clues obtained at the autopsy.

(a) Corrosive action along the gastro-intestinal tract may indicate alkali cyanides, ammonia, caustic alkalies, fluorides, heavy metals, mineral acids, oxalic acid, phenols.

(b) Odor of body cavities: alcohol, benzene, chloroform, cyanide, ether, nitrobenzene, opium, phenol, et cetera.

(c) Luminous particles visible in the dark, phosphorous.

(d) White (grayish) particles, arsenic; other colors may indicate copper, picric acid, et cetera.

(e) Seeds, leaves, and other such material may indicate an active drug.

THE POSTMORTEM EXAMINATION

In all cases of poisoning, a complete autopsy is necessary, not only to determine whether lesions produced by poisons are present, but to rule out natural causes of death. Best results are obtained if the body is fresh and not embalmed since the formaldehyde from the embalming fluid interferes with tests for cyanide, carbon monoxide, phenols, and the color reactions for the alkaloids. Putrefaction interferes with the detection of alkaloids. The presence of certain characteristic pathological changes is not always conclusive evidence of poisoning, because most of these changes (except corrosive action) are produced by various organic diseases. The pathologist can only say that the changes noted are such as might occur from a certain poison. We must also realize that some of the characteristic lesions usually produced by a definite poison may be absent.

The toxicologist should be present at the autopsy for the following reasons:

(1) To get leads or clues from the history of the case and from the autopsy.

(2) To supervise the handling, taking, placing in clean glass containers and sealing of the autopsy material. The containers must be clean and preferably of glass; the material should not be placed in rusty tin cans or wrapped in towels and newspapers. This is to avoid contamination and the loss of the volatile poisons.

(3) To get the weight of the various organs; this is absolutely necessary for the quantitative calculations.

(4) To see that the stomach contents and the intestinal contents are carefully saved and that they are not brought into contact with the internal organs when the stomach and intestines are opened for examination. If examination of the stomach mucosa is not necessary, the stomach should be tied at both ends with the contents intact before removing the organ from the body.

(5) To see that some blood is properly removed from the heart chambers for carbon monoxide determination. In cases of suspected drowning, blood must be taken from the left and right heart chambers and placed into two clean dry bottles, sealed, and properly labelled.

(6) To see that sufficient quantities of the proper organs, tissues and fluids are taken at this time. This will avoid the added expense and extra work of exhuming the body in order to get the needed material. As a general rule, the stomach and contents, part of the intestines and contents, liver, brain, lungs, kidneys, heart, some muscle, blood, spleen, urine, some bone and some hair should be taken.

(7) If the body has been embalmed, it is absolutely necessary to secure a sample of the fluid in order to determine whether or not the poison found in the body was introduced by the embalming.

(8) To see that a sample of water, in a case of suspected drowning in a pool, is obtained for the purpose of analyzing it for chloride content, since the chemical test for drowning depends upon the chloride content of the right and left heart chambers and it is essential that we know how much, if any, chloride was in the water of the pool.

(9) To see that a sample of air or gas is obtained if the deceased was found in a man hole or if it is suspected that death was caused by fumigation or to the inhalation of carbon monoxide.

If the toxicologist is not present, the autopsy material should be put in clean containers, properly sealed and labelled with the name of the deceased, date of autopsy, names of witnesses present, careful record being taken. The material should then be

sent to the toxicologist by a responsible person and a receipt taken for it. A record should be kept of the date of delivery, the person who made the delivery and the person who received the material. All this must be done to establish a direct relationship of all materials for court identification.

NECROPSY MATERIAL BEST ADAPTED FOR ANALYSIS

In the incidence of persons who come to hospitals on account of poisoning, the first stomach washing must be saved for analysis (not the eighth or tenth washing). Large volumes of urine, if possible, and all the feces excreted should also be submitted. Some blood should be taken for gas analysis. If found dead, or if the individual lived only a few hours after taking the poison, the stomach and intestinal contents are of importance in order to determine the poison quickly (acute case). The poison is most easily detected in the stomach contents in acute deaths. With the stomach and intestinal contents, the following tissues are of greatest importance: the brain for testing for alcohol, alkaloids, benzol, chloroform, ether, veronal group, et cetera; the liver for fluorides, metals, oxalic acid, sulphonal, veronal group; the kidneys for metals, especially mercury; blood for gases, such as carbon monoxide, and for the test for drowning; bones, hair and nails for arsenic, lead, radium; the lungs for inhaled gases, to prove whether poison entered by inhalation; and the urine for arsenic, mercury, sulphonal, veronal group.

Substances like acids, alkalies, ammonia, nitrates, nitrites, chlorates, sulphides, et cetera can be identified in the stomach and intestinal contents only, because as soon as these substances enter the circulation they are oxidized, reduced or transformed into normal constituents of the body and, therefore, cannot be detected in the internal organs. Some of these substances produced changes in the blood pigment (hemoglobin) and, therefore, the blood should be examined spectroscopically.

If the patient lives several days (five or six), the poisonous gases, volatile poisons and alkaloids cannot be found; they have been destroyed or excreted. The veronal group can be found as long as ten to twelve days thereafter. The metals can be found

for several weeks thereafter, especially in the bones, nails and hair.

THE TOXICOLOGICAL EXAMINATION

(1) The laboratory. Privacy is a first requisite. The laboratory should always be locked when the analyst is absent. This precaution is necessary to avoid the possibility of someone maliciously introducing poison into the autopsy material.

(2) The reagents must be of tested purity.

(3) Planning the analysis. No haphazard procedure should be tolerated. The analysis must be carefully planned before starting in order to save as much material as possible, for one can never replace necropsy material once it is completely used up. At least one-third of the total amount of material must be kept intact and properly sealed for the use of some other analyst to check the findings, if the court so desires.

(4) Weighing the total organs and measuring the volume of fluids received is essential in order to have all data available that are needed for the quantitative calculations.

(5) Examining the stomach contents for nature of food and time of digestion, for undissolved poisons, seeds, leaves, reaction, odor, color, et cetera.

(6) The chemical isolation of the poisons. Almost every toxicologist has a pet system of his own. I use the following classification upon which the chemical methods used to isolate the poisons are based.

(a) Poisonous gases, such as carbon monoxide, hydrogen cyanide, hydrogen sulphide, oxides of nitrogen.

(b) Volatile poisons, such as alcohols, acetone, fusel oil, aniline, benzol, camphor, chloral, chloroform, carbon bisulphide, croton oil, cyanide, formaldehyde, nitro-benzene, phenols, phosphorous, pyridine, et cetera.

(c) Acid-ether soluble poisons, such as acetanilid, antipyrine, barbituric acid group, benzoic acid, caffeine, cantharadine, colchicin, di- and tri-hydric phenols, oxalic acid, phenacetin, picric acid, picrotoxin, salicylates, santonin, sulphonal group.

(d) Alkaline-ether soluble poisons, such as aconitine, atropine, amines (poisonous), cinchonine, cocaine, codeine, cytisine,

delphinium, emetine, heroin, hydrastine, lobeline, narcotine, nicotine, pelletierrine, pilocarpine, piperazine, quinine, strychnine, taxine, veratrine, yohimbin.

(e) Ammonia-chloroform soluble poisons, such as heroin, morphine, apomorphine, narceine, papaverine, theobromine.

(f) Metallic poisons, such as antimony, arsenic, barium, copper, chromium, lead, mercury, radium, thallium, uranium, zinc.

(g) Mineral acids and caustic alkalies.

(h) Halogens and their salts, including fluorides.

(i) Salts of oxy-acids, borates, chlorates, nitrates.

(j) Poisons isolated by special methods: Adrenalin, anthraquinone, curare, drastic cathartics, ergot, glucosides, hypnotics containing bromine, muscarine, saponins, nitroglycerine, strophanthin.

(7) After the isolation, the qualitative tests are applied in order to identify the poisons.

(8) Having identified the poisons, fresh material is taken and the amount of poison in every tissue is quantitatively determined.

(9) From the amounts of poisons found in the various organs, the amount in the entire body is estimated.

(10) Report of analysis and findings should be submitted in thesis form as soon as the work is completed.

(11) The isolated poisons or the positive tests obtained should be sealed in glass vials and kept for court purposes as corpus delicti.

TOXICOLOGICAL WORK

Toxicological work is most difficult and great responsibility is attached to it. Every chemist knows how phosphorus or strychnine is detected, but to isolate and detect 0.25 mg. ($\frac{1}{400}$ grain) of strychnine mixed with 500 grams of tissue is an entirely different matter. Much experience is necessary. The person to whom the chemical analysis is entrusted should be a chemist with years of experience in the analysis of necropsy material, he should be accurate, honest and trustworthy; his integrity must be of the highest degree and, indeed, above the remotest suspicion because his evidence may be the basis for the acquittal of the innocent or the conviction of the guilty.

The toxicological experience in the Chief Medical Examiner's Office in New York City is the largest in the world, not overlooking the medicolegal institutes of Germany, Austria and France. In this office there are analyzed annually over 2000 human bodies. Since its inception in 1918, the Chief Medical Examiner's Office in New York City has analyzed over 25,000 human bodies for poisons.

In the United States there are only two localities in which toxicological work of this kind is done as a routine, namely, New York City and Newark, New Jersey.

While writing this paper, I was called by long distance telephone about a case that was troubling the authorities. A man was driving along a country road in an open car. Witnesses saw the car swerve off the road and stop. The driver was found dead. The coroner, without any scientific investigation, signed out the case as one of carbon monoxide poisoning. Doctors who had treated the man for a few years claimed that they advised him not to take the trip because of heart trouble. Now they were trying to argue it out, whether the man died of carbon monoxide poisoning or of natural causes. It would have been better to analyze the blood of this man in order to determine the presence or absence of carbon monoxide. Most of the cases throughout the country are bungled in this way.

Sometimes a case receives much notoriety in the newspapers. Then the authorities seek the services of experts who demand exorbitant fees and usually are not experts in this line at all. This condition exists throughout the country at the present time and was also prevalent in New York City before 1918. The experts in the Rice case cost New York City \$30,000 for trying to prove whether death was caused by chloroform or not. No analysis for chloroform was done. Instead, they argued it out in court. In the toxicological department of New York City a chloroform case of this kind would be completely solved in about two hours by chemical analysis. A rough estimate shows that the average cost to New York City for a complete toxicological examination, including grand jury and court testimony, is about \$5.00 per cadaver. In New York City today every case of ac-

cident, suicide, homicide and most of the sudden deaths with no medical attendance have necropsies performed and chemical analyses made.

It may not be out of place to depict a few typical cases in which toxicology played an important rôle in solving a crime or the cause of an accident.

Case 1. *Analysis of the stomach contents, the main, or perhaps the only mark of identification of the deceased. The Becker Case.* Becker and his wife and two children lived in the Bronx, N. Y. Mrs. Becker suddenly disappeared. Letters mailed in Philadelphia to friends of Mrs. Becker stated that she was tired of living with Becker and, therefore, she had gone to Philadelphia; further, that she was well, and not to worry any more about her. Her friends, however, did not believe these letters. They knew that, although she might have gone away from her husband, she loved her children too well to leave them behind. They, therefore, brought the matter to the attention of the District Attorney.

Investigation was started by the police. Mrs. Becker could not be found anywhere. After a few weeks of good detective work, a clue was unearthed, that Mrs. Becker had been buried in the yard behind a garage owned by a friend of Becker. The police proceeded to search for the body in this yard. After quite some digging up of earth, they found the body of a woman completely covered with lye. The clothes, face, and most of the external part of the body were badly erroded by the lye so that it was impossible to identify the body. Autopsy revealed that the woman's skull had been fractured by a blunt instrument. No poisons were found present on chemical analysis. The stomach contents revealed grapes, figs and nuts.

Meanwhile the detectives sought the person who had last seen Mrs. Becker alive. They found it to be a woman, a friend of the Beckers. She stated that Becker and his wife visited her about ten o'clock on this particular evening. When asked what they had eaten at her home, she told them, without knowledge of the chemical findings, that she had given them grapes, figs and nuts. It was mainly upon the finding of these particles of grapes, figs and nuts in the stomach that the State succeeded in the identification of the body as that of Mrs. Becker.

The Beckers left the house of this woman about 11:30 p.m., got into Becker's automobile and started for home. On the way Becker feigned engine trouble and drove into the aforesaid yard. He got out and lifted the hood. He called his wife to come out of the car and see for herself. As she stooped down to look, he hit her over the head, knocking her unconscious. He then threw her into a previously dug hole, threw lye all over her and buried her. He was tried and convicted, and paid the penalty.

Case 2. *A camouflaged poisoning case.* An automobile was found burning in an out of the way road. A man also in flames was lying across the front mud guard, dead. A case of this description would have been signed out by coroners as one in which death was due to accidental burning. The Medical Examiner's Office, however, investigated. A necropsy was performed and a toxicological analysis made.

It was found by scientific methods of analysis that the man did not die of the fire or the fumes or smoke. He was dead when the fire reached him. Further analysis revealed large amounts of cyanide in all his organs. Death was due to cyanide poisoning.

The case was solved as follows: This man had suffered great financial losses. He was practically penniless. He had a family and wanted to provide for them. He took out a large insurance policy with double indemnity in case of accident. His intention was to commit suicide and at the same time conceal it from the authorities in order to get the double indemnity. He set the car on fire and, when it was burning well, he drank a solution of cyanide. He then threw the container into nearby shrubbery and fell dead over the front mud guard. Thus a case which had the appearance of accidental death by fire was proved to be one of suicide by cyanide.

Case 3. *One that appeared to be suicidal gas poisoning was proved to be murder by suffocation. The Freindlich Case.* Freindlich, his wife and three children aged 9, 7 and 2 years respectively, lived in an East Side tenement in New York City. On the morning in question the father left the home at about 7 a.m. About 7:30 a.m. one of the boys smelled the odor of gas. He ran into

his mother's room and found her in bed. He shook her but she did not respond. The boy called for help. The neighbors called the police who in turn notified the Medical Examiner's Office. On arrival, the Medical Examiner found that the room was filled with illuminating gas; the gas jet was open; the woman was dead in bed, lying on her back in a natural position. There was no pink coloration of the skin of her face or of any other part of her body (as is usual in carbon monoxide deaths). Lying in a crib a few yards away was the two year old baby, still alive. The baby was quickly removed to fresh air and saved. The absence of pink coloration of the skin of the woman and the fact that the baby was still alive and the mother dead, made the Medical Examiner suspicious and a necropsy was performed.

Toxicological analysis of the blood removed from the heart of the deceased mother showed complete absence of any carbon monoxide. This indicated that death was not due to inhalation of illuminating gas. The necropsy also showed that death was due to suffocation. Ten finger marks of compression were found on the back of her neck.

Freindlich murdered his wife for insurance money. He held her face down against the pillow until she was dead. He then turned her on her back, straightened out all the bed clothing, turned on the gas and left the house. He was tried and convicted. Coroners or inexperienced county physicians would have signed out the case as suicide by inhaling gas, whereas it was murder by suffocation.

Alcohol the cause of accidents

The toxicological laboratory of New York City quantitatively analyzes the brain for alcohol in all cases of fatal accident, the purpose being to determine whether alcoholic intoxication was a contributory cause to the accident.

Research work has been done in this laboratory on over 6000 human brains as well as on the spinal fluid and blood of living human alcoholics, and many series of experiments have been carried out on dogs in which brains, blood and spinal fluid were analyzed. From these experiments, it was determined that if the alcoholic

content of the brain or of the spinal fluid reaches above 0.25 per cent, it indicates that the individual was intoxicated. This was found to be true whether the individual was an abstainer or a habitue.

Case 4. *Case of a famous air pilot.* This flier was one of the first few who successfully crossed the Atlantic. Several years later he took off at Roosevelt Field with two male occupants. Within a very few minutes the plane crashed to earth and all three were killed. Examination of the wrecked plane revealed nothing as to the cause of the accident. A necropsy was performed on the deceased pilot and his organs chemically analyzed. The results of the analyses indicated that the pilot was intoxicated at the time he took off and evidently his condition of intoxication was the contributory cause of the fatal accident.

Case 5. *Planted body for purpose of collecting insurance.* This is the story of two undertakers who were partners in their business in a rural community. One of the undertakers took out a large life insurance policy with double indemnity for death due to accident and made his partner the beneficiary. Not many months later a bungalow that they owned was burned to the ground. The burnt body of a man was found in the ruins. The undertaker who was the beneficiary claimed that the body was that of his partner, and put in his claim for the insurance, asking for double indemnity because the death was caused by accidental burning. The insurance company started an investigation. A necropsy and a toxicological investigation were conducted. The results of the investigation revealed the following:

(1) The external part of the body was completely charred. The head was completely burned off. The feet and part of one leg were burned off. No identification was possible.

(2) By measurement of the bones of the extremities that were left, it was estimated that the deceased had been about two inches taller than the missing undertaker.

(3) Some parts of the internal organs were still in good shape, that is, they were not burned or boiled. Examination of the lungs showed a well developed pneumonia, whereas the undertaker in question was seen in apparently perfect health only two hours before the fire.

(4) Chemical analysis revealed no poisons, ruling out suicide by poison.

(5) Chemical work further revealed that the body found in the ruins had been dead when the fire started. The fire or fumes or smoke had nothing to do with this man's death.

(6) Toxicological analysis also revealed the presence of formaldehyde in the various organs. This strongly indicated that the body had been embalmed.

It was finally determined that the body was not that of the undertaker. It was a man two inches taller who had died of pneumonia and had been embalmed for burial. This body the undertakers planted in the house and then set it afire.

The five cases above outlined illustrate the kind of work the toxicological department of the Medical Examiner's Office in New York City is doing.

In conclusion, it will not be out of place to call attention to the National Research Council's Bulletin Number 64 (Washington, D. C., p. 75, 1928), where the following statement is made concerning the work of the Chief Medical Examiner's Office of New York City:

In the scientific attainment and administrative ability of its chief, in the character and volume of the work done, in the working facilities and budget allowed it, in its functioning in the investigation of crime, in its cooperative relation to other agencies, in the service which it gives to the public, and in its freedom from political interference, the office of the Chief Medical Examiner of New York City is the most outstanding agency devoted to forensic medicine in the United States.

DISCUSSION

WM. D. McNALLY, Chicago, Illinois: Toxicology is a broad subject, it treats of poisons, their origin, properties, physiological action, treatment of their noxious effects and their detection by chemical or other means. The toxicologist is a scientific detective looking for clues as to the rôle played by poisons in causing death.

Dr. Gettler spoke of the use of embalming fluids interfering with the detection of poisons. Where a body has been embalmed, a portion of the fluid used should be examined by the chemist for

the presence of poisons. The lack of this precaution, placed a pathologist and chemist in a near by state in a most embarrassing position.

A man was found early one morning in a small car, which was wrecked against an abutment on the highway. He was taken to a hospital. Late that afternoon he said he felt well enough to go home and was released by the hospital. The following morning he died at his home. An autopsy was held by the local coroner, who gave as the cause for death: "ruptured stomach." The undertaker prepared the body for burial. Late in the afternoon an insurance company wanted a second postmortem which was granted. The liver, kidneys and stomach were removed for chemical and pathological examination. The insurance company a short time later received a report that "death was due to arsenical poisoning."

The man was a crank on life insurance and had taken out over \$140,000. Every policy was contested. The family employed counsel. He came to Chicago and wanted assistance. The family knew the deceased did not commit suicide. Drug stores in neighboring towns were canvassed, no purchase of arsenic could be found. I requested specimens of the embalming fluids and hardening compounds used after both postmortems. The samples were received, analyzed and a short telegram dispatched, "Mystery solved, letter to follow." A few weeks lapsed and a lawsuit was started. The insurance company introduced the pathologist who testified that he found grossly and microscopically, evidence of arsenical poisoning. The chemist reported 15.4 grains of absorbed arsenic in the stomach, arsenic in the liver and kidney. The plaintiff introduced the undertaker who had embalmed the bodies and submitted samples to me for examination. Bottles of embalming fluid and a large jar of hardening compound contained over 50 per cent of arsenic trioxide. This compound had been used after the first autopsy, the organs covered with this arsenic compound had been removed and reported as the causal agent for death.

Of course the widow received all of her insurance.

We hear frequently that aviators are half intoxicated when

entering their ship. Dr. Gettler's analysis of the organs of an aviator killed while driving his plane appears to bear that out. In one fatal case in Chicago, I found carbon monoxide in the blood of the pilot.

In Chicago, in 1913 I established under Coroner Peter Hoffman the first Coroner's laboratory in the United States which was several years ahead of the establishment of the laboratory of the Medical Examiners in New York.

In Cook County we can not equal the price of \$3.00 per toxicological examination as given by Dr. Gettler. The salary of the chemists and the overhead expenses would bring the cost above \$3.00 per case.

I dislike very much the reporting of alcohol by the plus sign, one plus, two plus or four plus. As each one of these indicates a certain percentage of alcohol, I believe that the percentage should be used.

Dr. Gettler spoke of clean glassware being used for submitting specimens and I would like to add that metallic tops to jars should never be used, as arsenic and lead are frequently found in the metallic tops giving an opportunity for defense attorneys to claim contamination of the organs examined.

Manufacturers of chemicals and drugs are always a jump ahead of the toxicologist and it is necessary to develop methods of detection for each new group of drugs produced. Some poisons escape detection because of lack of delicate methods or because of chemical changes that these drugs undergo in the body or after embalming.

MEDICAL EXAMINERS' FINDINGS IN DEATHS FROM SHOOTING, STABBING, CUTTING AND ASPHYXIA

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PART I. DEATH FROM SHOOTING

HOMICIDAL SHOOTINGS

Shooting has always been a common method of committing murder. It apparently reached its highest peak of popularity in the United States and incidentally in the world, during the last few years of our recent prohibition era, when it became the chief method of killing amongst gangsters, racketeers, hi-jackers, hold-up men, et cetera.

During an eight year period (1925-1932) in Essex County out of 360 homicides, 167 were killed by shooting, constituting 46 percent of the total number.

In 2,457 homicides in New York City during a five year period (1928-1932) 1,386 lost their lives by shooting or 56 percent of the total murders.

Homicides by shooting, therefore, form at least 50 percent and over of the total murders occurring in and around the metropolitan district of New York. The methods of killing next in frequency are stabbing and cutting, assault and battery, and strangulation by ligature.

In main, the popularity of shooting lies in the fact that close contact with the victim is unnecessary, the danger of a fight is avoided and the automobile usually affords a quick getaway.

SUICIDAL SHOOTINGS

Shooting is also a common way of committing suicide.

In 1,056 suicides in Essex County during an eight year period

(1925-1932) there were 172 suicides by shooting or 16 percent of the total suicides.

In 7,219 suicides in New York City during a similar five year period (1928-1932), 694 or 10 percent of the total suicides were due to shooting.

Suicides by shooting, therefore, form only about 10 percent of the total suicides occurring in and around the New York district. The commonest methods are the inhalation of illuminant gas, hanging, jumping from buildings, shooting, poisoning, stabbing and drowning, in order of frequency.

ACCIDENTAL SHOOTINGS

In large cities accidental shootings are not common. They occur especially among boys playing with firearms.

WAR WOUNDS

The familiar GSW's of the World War forming the major part of the battle casualties, produced by fragments of high explosive shells, by machine gun and rifle bullets, hand grenades, et cetera, will not be considered here.

TYPE OF WEAPONS USED IN CIVIL WOUNDS

In suicidal shootings the commonest weapon is the *revolver*. This is usually a cheap model, in poor or neglected condition, of .32 or .38 caliber, discharging a lead bullet of low velocity. Often, however, a good revolver or an automatic pistol of .25, .32, .38, or even .45 caliber is used. Occasionally, a shotgun or a rifle is the weapon of choice.

In the ordinary gin mill and lust murders cheap revolvers are usually employed. Amongst gangsters better revolvers, automatic pistols and often high velocity weapons such as machine guns are used.

In accidental shootings the weapon is often an automatic pistol, —a dangerous weapon in untrained hands since the barrel often contains an unsuspected cartridge. Among hunters accidental shooting with shotguns and rifles are all too common.

VIEWING BODY AT SCENE OF DEATH

In all shooting cases when a dead body is found, it is of prime importance that the scene be visited by the medical examiner and that nothing on the body, or in its immediate vicinity, be touched until his arrival.

In New York City and Essex County, the medical examiner under the law "must go to the dead body and take charge of the same." In all homicide and suicide cases, the viewing of the body at the scene of death by an experienced medical officer is of vital importance. The medical examiners in these areas, upon arrival at the scene have precedence over all other individuals and agencies, and until his medical investigation is completed and he has selected and taken possession of such objects as he decided may aid him in his investigations, no one, not even the police, may take charge of or remove the body or any object whatsoever.

Effective coöperation with the police is absolutely essential, and under the medical examiner's system the police and medical examiners work as one unit toward a common cause—the detection of crime and the fixing of criminal negligence.

In suspected murder cases the homicide squad often arrives at the scene before the medical examiner they are, however, trained to touch nothing until his arrival. Then all work together, the photographer, finger print man, et cetera. A careful inspection of the body and its surroundings is made noting everything that might assist in determining whether the shooting was accidental, suicidal or homicidal. In the event of murder everything, which may lead to the detection of the murderer, is noted.

Proper instruction of the police and ambulance surgeons soon prevents the careless picking up of a weapon if found, or other important clues.

If there is any doubt as to whether the victim is dead, any necessary disturbance of the clothing or moving of the body for the purpose of determining this by ambulance surgeons or physicians called in in the emergency is, of course, excusable.

Notes should be taken, on the spot, by the medical examiner to his stenographer, or in long hand when a stenographer is not avail-

able as to the position of the body, its temperature, the absence or presence of rigor mortis, how the body is dressed, and the location of the wounds. The body should not be moved too much for this purpose until photographs have been taken.

Absence of the weapon, usually means murder, but a careful search must be made, since suicides, after wounds which would apparently indicate sudden death, may throw the gun out of the window or in a dark corner.

Furthermore, the presence of the gun in or near the hands of the dead man, though usually indicating suicide, may have been placed in the hand of the dying man by the murderer.

Search should be made for powder marks or burns on the hands of the deceased, since often in suicide by means of cheap revolvers, a flare back may occur from the chamber of the gun. Powder marks or burns on the hands do not always indicate suicide since they may have been produced in a struggle with the murderer.

In the examination of the wounds it is sufficient only that their main characteristics be described, and an attempt be made to determine chiefly whether they are wounds of entrance or exit, since their closer examination will take place at the morgue under better light and circumstances.

Careful search should be made for empty shells, ejected from either automatic pistols or repeating rifles; they are usually found in the immediate vicinity of the weapon or near where it was fired.

Spent bullets may often be found on the floor or under the body, and search should be made for embedded bullets in plaster, wood-work, furniture, pillows, and other objects, when necessary.

The gun if found should be carefully picked up, preferably with a clean handkerchief, so as not to disturb finger prints, and unloaded. During the unloading the muzzle should never be pointed towards anyone and the medical examiner should acquaint himself with the proper method of breaking and unloading the various types of firearms, especially the automatic pistol.

Except in cases of clean cut suicide, the body should be photographed as well as the surrounding rooms or location. After the police photographer has finished with the body it may either be reexamined more closely at once or later at the morgue.

Personal effects and identification cards, letters, et cetera, are usually removed at this time by a thorough search of the clothing as this usually gives police early clues to work on.

The body is then sent to the official morgue at which place it must not be disturbed or undressed until the arrival of the medical examiner.

THE NECROPSY IN SHOOTING CASES

Undressing the body

The body should be undressed by the medical examiner with the assistance of the morgue keeper, or by the morgue keeper under supervision. Care must be taken to watch for bullets that might drop from the clothes; they are frequently found between the skin and clothing, lacking the force to come out through the clothes. The same precautions should be taken in undressing wounded men in the receiving rooms of the hospitals.

It is a good plan to start with the shoes and work up, describing the condition of the various articles of clothing to the stenographer, as they are removed. Holes made by bullets entering and leaving the clothes should be carefully described, especially the holes of entrance in the outermost garments where clinging powder grains and burns may readily be seen. If found, the clothing should be carefully folded so that this evidence will not be lost. Clothing in all murder cases should be saved and properly marked for the crime detection bureau of the police or for the district attorney.

IDENTIFICATION OF BODY

Proper identification of the body must be made in all important cases before the necropsy begins. If the medical examiner has seen the deceased at the scene of the shooting he can identify the body he is about to autopsy as the one he examined at place of death.

If the medical examiner has not previously seen the body, especially in cases dying in hospitals, an identification must be made in his presence by the police officer who brought the victim

to the hospital, or by relatives or friends of the deceased. This should be taken down by the stenographer as a part of the necropsy protocol, giving in detail the names and addresses of those who identify body.

The Necropsy

After the body has been undressed and properly identified, the necropsy should be conducted in a careful routine manner. All wounds of entrance and exit should be carefully examined and described. In examining wounds of entrance care should be taken not to wash off powder smudge or partly embedded powder grains when cleansing the wound of blood. The wounds should then be photographed. It is good practice to measure in inches* the distance above the heel of every wound of entrance and exit. The height of the wound of entrance and exit above the heel will often make it possible to reconstruct the direction of the shot and to tell on what line the gun was when fired.

Before starting the internal examination of the body it should be turned and the back examined. Bullets of .32 or .25 caliber do not usually possess sufficient velocity to pass through the body and can often be felt underneath the skin of the back. They are frequently surrounded by a small hematoma.

The necropsy is then performed in the routine manner. For years we have used the ordinary Virchow technic in performing both scientific and medicolegal autopsies, and seldom find it necessary to remove the organs of the chest and abdomen "en masse." We believe that many points of importance may be missed in removing organs in toto, as the amount and character of the blood in right and left heart cavities; the presence of fat emboli; the condition and contents of the bronchi; the presence of pulmonary embolus and its dislodgement, et cetera. We prefer removing one organ at a time, describing lesions as encoun-

* In medicolegal work we prefer the use of inches, having entirely given up the metric system as applied to measurements. Centimeters mean nothing to an American jury and probably never will. The weights of organs can still be taken in metric system as they are not often needed in court and can easily be transcribed.

tered. The course of a bullet through the body is seldom lost since it is always marked by a path of hemorrhage.

The wound of entrance and exit in all the organs are described in the individual examination of each organ and the direction and course of a bullet is finally summed up after it has been traced from entrance to exit, or to the location where it was found.

If a bullet is found loose in the pleural, peritoneal or pelvic cavities, or in the subdural space, the location where it was found cannot be taken as a fixed point since it may have shifted to this position by gravity. The finding of a bullet over the left occipital lobe may result from the reclining position of the body after death, and the real point of exit in the brain may be over the left frontal lobe where the point of impact may often be noted on the interior of the calvarium by the presence of a bursting fracture.

When, however, a point of exit is found in the spine or in the posterior thorax, provided the bullet has not been deflected by striking bone previously, this point of exit when joined by a large probe with the point of entrance, gives a good idea of the general direction of the bullet, and it is good practice to measure how far the fixed point of exit is behind, below, or to the right or left of the fixed point of entrance.

Furthermore, the actual hole of entrance in the skin is often not a fixed point for measuring, for instance, when it is found in the arm or in the region of the female breast. The skin wound may not correspond to the wound of entrance in the chest wall in the prone position of the body at necropsy unless the parts are placed in the same position as they were when the shot was fired.

Great difficulty may arise when two, three or more shots enter the body in near proximity or their tracts cross each other in the body. The course of different bullets often crisscross each other and considerable experience is needed to properly interpret the correct course of each individual bullet.

When a bullet after passing through thoracic or abdominal organs, finally leaves the body cavities and becomes embedded in the back, spine or pelvis, the tract may often be lost, since there may be very little hemorrhage in a bullet tract through bone. Much time is often saved by fixing the point at which the bullet

has left the internal cavities of the body, by turning the body over in a position to cut down on the overlying muscles, and other structures. Hemorrhage will quickly be found, if the bullet has passed through to the back, by using large clean cuts and following them up to the location of the missile. Where there is no hemorrhage there is no bullet.

There is no place in the body where a bullet may not lodge and sometimes it becomes necessary to search for hours unless x-ray facilities are at hand. Sometimes the bullet may be deeply embedded in the body of a vertebra, and may be entirely missed by lying $\frac{1}{4}$ of an inch beyond a saw cut. In such cases a great deal of time will be saved by examining the body or certain portions of it by means of the x-ray.

It is surprising that practically no necropsy rooms in this country are provided with x-ray equipment. We have x-ray facilities, but it is often inconvenient to transfer a dead body partly examined to an x-ray department and ask for certain examinations. It would be of great value to the medical examiner in locating bullets, and certainly to the hospital pathologists in the study of bone diseases and skeletal metastases to have x-ray equipment in or adjacent to the necropsy rooms. It would be particularly useful in this country where the examination of the skeleton at necropsy is, for obvious reasons, a very limited one.

Occasionally bullets cannot be found in cases in which there is no apparent exit from the body. Some of these cases have spat up or coughed up the bullet; others may have been swallowed and lost in the stool, or, rarely, found in the intestinal tract.

The necropsy should be completed with great care and a verbatim dictation typed into a protocol. This should be signed by the medical examiner who performed the necropsy. It should be filed as an official record in the office of the chief medical examiner. Special anatomical charts of the surface and different parts of the body are useful for charting the location of the wounds. Microscopical, chemical or any other examinations that are necessary should be completed, or the specimens taken before the body is released for burial.

Marking bullets for identification preservation

All bullets must be properly marked and kept safely for future identification, study or for trial. It is very difficult to properly mark bullets, since practically only the butt is available. The riflings on the sides must be left intact for the ballistic expert. If the butt is flat, an identification mark, a number or initials may be marked by the use of a small jeweler's die or tool. The difficulty is that there is room only for a small symbol, and when handling a large number of bullets, this means very little in the way of identification.

If the butt is deeply conical it is practically impossible to mark a small bullet. I have had my identification mark on such a bullet covered with wax used by the ballistic expert in fastening the bullet to the comparison microscope. Later at the trial I was chagrined at being unable to find any mark until the wax was removed.

For these reasons we do not mark bullets except in unusual circumstances. We prefer keeping them in sealed envelopes containing a description of the bullet with other important data in the handwriting of the medical examiner. If the bullet is needed by the prosecutor it can be obtained on receipt by a responsible officer, who later returns it to the medical examiner. At the trial the bullet is introduced by the medical examiner when testifying as to the cause of death. This prevents much wrangling.

REVOLVER WOUNDS

Revolvers usually shoot lead bullets of low velocity, 640 feet per second being about the maximum speed. The cartridges which formerly contained black powder are now frequently loaded with smokeless powder. This is of prime importance since the blackening and tatooing described in most textbooks is either insignificant or not present at all in many revolver wounds seen at the present time, due to the almost complete combustion of smokeless powder. Smokeless powder burns more rapidly, produces greater velocity and does not smoke or foul the barrel.

It is also possible to load revolvers with jacketed ammunition designed for automatics. This, however, is not common. Prac-

tically all revolver and pistol ammunition is now center fire. The old rim fire is seldom seen except in small pistols and rifles used in shooting galleries.

The calibers more commonly used in revolvers are .22, .25, .32, .38 and .45. These calibers are used in automatic pistols and rifles also. The caliber of a cartridge or gun is designated in hundredths of an inch. For instance, when we speak of a "Colt .38" we mean a Colt revolver of .38 caliber which takes a cartridge having a diameter of 38/100ths of an inch.

Continental firearms are, of course, calibrated according to the metric system.

While the cartridges for a .38 caliber Colt are made for this gun by the manufacturers, they can be used in any other revolver of the same caliber. They can also be used in automatic pistols, repeating rifles and machine guns of the same caliber. Thus, from the size of a bullet and shell alone, one cannot tell whether a revolver or rifle was used.

Contact revolver wounds

When the muzzle of a .32 or .38 caliber revolver is held against the skin and fired, the wound of entrance is usually larger than the diameter of the bullet. Frequently it is a lacerated, stellate or cruciform wound which does not resemble a bullet wound in any way.

This is due to the fact that the gases from the explosion enter the wound and cause subcutaneous excavation, often tearing the skin wound, the subcutaneous tissues, and producing a large blackened hole or laceration. There may be slight burning (brand) about the wound, but since the gun cannot kick there is no particular location of the brand. Blackening due to a powder smudge which can be wiped off, may show around the edges of the wound and may result in slight tattooing (embedding of unexploded or partly combusted powder grains) in the skin about the wound.

However, if the gun is tightly pressed against the skin, as it often is in suicide and gangster murders in administering the coup de grâce, there may be no marks in the surrounding skin,

and the wound of entrance may be seen as a large, torn, black hole with a charred bed.

If the body is protected by clothing over the wound of entrance, the clothing often shows a cruciform tear with firing of the clothes. This firing may produce quite an area of burning in the underlying skin.

If the bullet passes through the body without striking bone or without too much loss in velocity, the wound of exit is often a round hole about the diameter of the bullet with everted edges, the wound of entrance being larger in these cases than the wound of exit.

Near contact revolver wounds up to six inches from body

If the revolver is fired near the skin the wound of entrance may resemble a contact wound. If fired at a distance of one to 6 inches from the body, the shape of the wound is usually round or oval and approaches the diameter of the bullet, depending on the angle at which it strikes the skin. In such cases a brand or area of burning caused by the escape of explosive gases from the muzzle of the gun may be seen about the wound. This brand is often concentrated in an oval area most marked over one side of the wound. This is caused by the upward kick of the gun when fired and is good evidence that the trigger of the gun was in a position opposite this mark. Scorched or burned hair may be found in this area.

A sharply demarcated area of blackening or smudge may be noted around the wound, often covering an area about one inch in diameter. It is caused by a powder smudge and can be wiped off.

Tatooing caused by the embedding of incompletely burned powder grains in the skin may be seen around the wound. This may be extensive when black powder is used. Ten to 100 or more grains may often be counted. The tatooing may extend quite a distance from the wound. For instance, in a bullet wound of the cheek, powder burns may be noted over the concha of the ear and may denote the direction of the shooting. Flakes of powder resting on or slightly embedded in the skin are easily dislodged

and care should be taken in cleansing the skin, or in folding the clothes, not to destroy this evidence for further examination.

With smokeless powder, very little blackening or tatooing may be present and a hand lens is often useful in the examination of such wounds.

Revolver wounds made at a distance of six to twelve inches from body

When the muzzle of a .32 or .38 caliber revolver is held at a distance of from six to twelve inches from the body and fired, the bullet wound of entrance is usually about the size of the bullet. There is no tearing or laceration of the skin. The bullet hole is usually round with its margins bruised and its edges inverted when the angle of fire approximates a right angle to the surface of the body. If the bullet strikes the body at an angle, the wound may show a tangential bruising to one side, the so-called slap shot. At this distance the brand or burning is usually absent, or if present, slight in extent. The wound still shows a small amount of blackening or powder smudge as a rule, especially if black powder is used. Tatooing may still be present but is usually small in amount. There is no firing of the clothes at this distance.

Revolver wounds made at a distance of two to three feet and over

At these distances the wound of entrance is a round hole about the size of the diameter of the bullet. The edges are often slightly inverted and may be slightly discolored from the lead or grease of the bullet. There is usually a thin contusion collar about the wound, the skin being abraded or bruised by the lateral walls of the bullet as it penetrates the skin.

If the bullet strikes a body at an angle, the wound of entrance is a round or slightly oval hole often with a tangential bruising or abrasion on the skin, denoting that the bullet has entered at an oblique angle, an important point in estimating the direction of fire.

The brand or burning, the blackening or smudging and the tatooing at these distances have entirely disappeared. The clothes simply show a round or lacerated tear of entrance and are free from burning or powder.

Can the caliber of the bullet be estimated from the size of the wound of entrance?

Except in contact shots or near contact shots in which the wound of entrance is often larger than the bullet, all shots at greater distances produce, as a rule, a round hole of entrance which approximates the size of the bullet. Due to the elasticity of the skin, however, an accurate estimate of the caliber of the bullet cannot be made. Roughly we may state that wounds made by .38 and .45 caliber revolvers are usually one-fourth of an inch or more in diameter, and those made by .32 and .25 caliber guns are a little smaller than one-fourth of an inch. If the victim lives a few days the dried scab and retraction so distorts the wound that no estimates can be made.

Wounds of exit

Except in contact shots or near contact shots in which the wound of entrance is often larger than the wound of exit, when a revolver bullet passes through the body, losing little in velocity and failing to strike bone, the wound of exit may closely simulate the wound of entrance in size. Usually the exit wound is a trifle larger, its edges slightly everted, and sometimes a strand of tissue may be seen hanging from the wound. Sometimes it may be quite difficult to determine a wound of exit from one of entrance, especially if the person has lived a few days. Of course, there would be no evidence of burning, smudging or tatooing about the wound of exit.

Often one must resort to a careful examination of the clothing to distinguish between wounds of entrance and exit, the examination often settling the question. Burning and firing of the clothes without the underlying skin being affected may show the wound to be one of entrance. Strands of clothing may project externally around a hole in a coat signifying an exit.

Revolver bullets of .38 and .45 caliber often pass through the body, while those of .32 or smaller caliber are slowed up and their velocity so reduced that they frequently remain in the body. They are often found beneath the skin of the back.

Bullets which pass through the body with loss of velocity on

account of the greater distance fired, or from the resistance of penetrated tissues, or because they were fired from poor guns, usually produce wounds of exit which are much larger than the wounds of entrance. They are easily diagnosed and often show extensive tearing with everted edges.

The bullet usually remains in the body if bone is struck. If it leaves the body it often produces a large hole of exit due to loss in velocity, splitting or mushrooming the bullet, or to some spicules of bone blown out with the bullet. Sometimes more than one hole of exit is thus produced.

In passing through the skull a bullet often makes a wound of entrance consisting of a sharply cut round hole beveled on the inner table, while the wound of exit is much larger and is beveled externally. This is characteristic and may sometimes, in an exhumed body when the wounds in the soft parts have been obliterated by decomposition, settle a question of murder or suicide.

Deflection of bullets

Ordinarily bullets are not deflected from their course in passing through the body unless they strike bone. Therefore, if a large probe can be passed through the wound of entrance and out the wound of exit, it usually denotes the direction of the bullet through the body. A continuation of this line from the wound of entrance shows the line at which the barrel of the revolver was when the shot was fired. This is all one is safe in testifying, unless one has witnessed the murder and knows the actual position of the body at the time.

AUTOMATIC PISTOL WOUNDS

Automatic pistols fire a jacketed bullet of high velocity attaining a speed of from 800 to 1,200 feet per second. These bullets are often spoken of as "steel jacketed." This is erroneous since a steel jacket would ruin the rifling of a gun. The jacket is usually of copper or cupronickel, some of the recent ammunition being covered by a plating process. The cartridges contain smokeless powder.

Wounds produced by automatics do not possess all the characteristics of revolver wounds and are much more difficult to interpret. Absence of burning and discoloration in close discharges are common, and the wound of entrance must often be examined with a hand lens, and even chemical examination of the edges may be important. There is much greater penetration with such weapons. Otherwise both wounds of entrance and exit are the same as for revolvers.

MACHINE GUN AND RIFLE WOUNDS

Accidental shooting by means of a small .22 caliber rifle occasionally occurs in boys playing, or taking "pot shots" at electric lights or windows. In rare instances such a weapon may be used for committing murder. Such wounds do not produce much damage unless they penetrate some vital organ producing internal hemorrhage, peritonitis or infection. While apparently insignificant they are often tricky and produce unexpected damage which is fatal.

Larger rifles are occasionally used in committing murder and suicide. Accidental shooting with high powered rifles in some parts of the country is common, especially in the hunting season.

Gangsters frequently use machine or submachine guns in "bumping off" their victims.

Practically all of these weapons shoot bullets of high velocity, often attaining a speed of from 2,000 to 3,000 feet per second.

At short range up to 200 yards, the rifling of the gun barrel rotates the bullet about 2,500 revolutions per second and causes an eccentric whirling of the butt. This produces extensive bruising of the deep tissues and tremendous fragmentation of bone.

With high velocity rifles, the wound of entrance is often smaller than the diameter of the bullet. The edges are depressed with a reddish zone around the circumference which becomes brown on drying. If the bullet strikes at an angle, the skin may be split or extensively ploughed up.

At medium range, 300 to 1,000 yards, the tissues are cleanly cut and a clean hole is made in the bone. The eccentric whirling of the butt largely disappears.

At long ranges, 1,000 yards or over, the bullet loses so much velocity that extensive wounds of exit may result.

SHOTGUN WOUNDS

All shotguns are now more or less choke-bored, that is the barrel at the muzzle is smaller in diameter than at any place behind the muzzle except the chamber. Barrels, therefore, may be full choke, half choke, one-quarter choke, and of the old cylindrical bore. The rate of dispersion of the shot depends on the amount of choking in the barrel.

Cartridges used in shotguns are first loaded with powder, on top of which is placed a cardboard and two or three felt wads. The shot is then placed on these wads and held down by a single cardboard wad, over which the edge of the cartridge shell is crimped.

The shot used is either soft lead or chilled and is designated according to size, as buckshot, and numbers, 2, 4, 5, 6, 7, 8 and 9, the lower the number, the larger the size of the pellet.

Shotgun wounds made at close range

When an ordinary shotgun is fired a few inches from the body, the entire charge, often including the wads, enters the body "en masse" producing a large ragged hole with extensive tearing of the underlying tissues and rupture of the underlying viscera. The hole is blackened, burned and tattooed, often with powder driven deep into the wound. Frequently in a shotgun wound of the chest, wadding and bits of clothing may be found lying against the spine, together with firing of the clothes.

At the distance of one yard the shot still enter the body as one mass, but the hole is more regular in outline, usually round and about one inch in diameter. Firing of the clothes is common.

Shotgun wounds made at 2 to 4 yards distance

At a distance of two to three yards the wound of entrance is still a ragged hole with most of the shot entering the body en masse. However, there is a beginning scattering of individual shot, some striking and entering the body usually above the top

of a large wound, due to the upward recoil of the gun. There is no burning or blackening at these distances but a certain amount of tatooing may still be seen.

At the distance of four yards, dispersion has reached such a degree that the shot enter the body as individual pellets covering a square pattern about six to eight inches in diameter. Many of the shot enter the body in small groups.

Shotgun wounds made at ten yards distance

At ten yards the shot enter as individual shot without any grouping and cover a square pattern about twenty inches in diameter.

Shotgun wounds in general

Naturally the above examples are to be used only as a general guide in the approximate determination of the distance the shotgun was fired. Each individual case, especially if it turns out to be an important one in which the life of an innocent man may be at stake, should take into consideration the exact gauge of the gun in question, the amount of choking of the barrel, the type of ammunition used, et cetera. Test shots and a complete study of the situation by a ballistie expert may be necessary.

The dispersion with fully choked barrels is about one half of that given in the above examples. With smokeless powder there is much less blackening and tatooing, the powder stains usually being greyish in color.

In general, shotgun wounds may be homicidal, suicidal, or accidental. Sometimes a wound apparently insignificant because of its size and location may produce death from internal hemorrhage. In suicidal shootings the trigger may be pulled in a variety of ways, some of which are often ingenious. For instance, the gun may be fired with the foot by using a bent piece of wire laid over the trigger and by many other devices.

SUMMARY

The most important question, of course, for the medical examiner and police to determine is whether the shooting was murder, suicide or accidental.

Herzog (sec. 282, p. 234) states

The question whether death was due to murder or suicide arises frequently and often even the closest and most careful examination will fail to determine it, as persons in committing suicide frequently cut and wound themselves in such revolting and barbarous ways that the corpse and its surroundings present the appearance of murder should no more bias the examiner toward that conclusion than that a case presenting all appearance of suicide would cause the examiner to conclude that he has to deal with a case of suicide; for even as a suicide may in his suicidal rage unwittingly, or perhaps because his insurance policy has not yet run long enough to make it incontestable in case of suicide, give his death the appearance of accident or murder in order to entitle his beneficiary to the insurance money, even so a murderer may and will often, to avoid suspicion, do everything possible to make the murder committed by him appear a case of suicide or accident.

Persons committing suicide by shooting or by cutting their throats, frequently stand in front of a mirror.

The location of the wound often affords considerable evidence as to whether the wound was homicidal, suicidal or accidental.

Suicidal wounds are usually situated in the front of the body or in those parts most easily accessible. In right handed persons the bullet wound of entrance in the head is usually in the region of the right temple or in front of the ear; in left handed persons the wound is on the left side. Occasionally the wound may be found in the roof of the mouth.

Homicidal wounds may occur in any part of the head but are most commonly found on the back of the head, the shot usually being fired from the rear. In victims taken for a ride the coup de grâce is often found in the back of the head near the external occipital protuberance, the shot being fired by the gangster sitting in the back seat of the automobile and the gun placed against the base of the skull.

Some parts of the body cannot be reached at all or only with difficulty by a suicide, thus wounds in the back are usually homicidal.

Although the person who has decided on self-destruction usually adopts the methods which are obvious and which he believes will result promptly in death, there are many cases reported in which very unusual means are employed, so that one must not be misled by the type or situation of the wound.

Taylor⁹ states

There is no wound which a suicide is capable of inflicting on himself which may not be produced by a murderer; but there are many wounds inflicted by a murderer which, from their situation and other circumstances a suicide would be incapable of producing on his own person.

Was death instantaneous?

Herzog (sec. 294, p. 242) states

In many instances the amount of blood surrounding body will show whether the person bled to death; the appearance of the wound will show whether death was instantaneous or almost so. Thus for example, where a person places the barrel of a gun into his mouth and pulls the trigger, blowing off half of his head, there can be no doubt that death was instantaneous, but a tiny hole in the center of the forehead, caused by a .22 caliber rifle bullet does not negate the possibility that the dead person may have done some voluntary act after receiving the wound.

Contrary to lay and some medical opinion, a bullet wound of the heart does not always produce immediate death. After such a wound it may be possible for the victim to walk a considerable distance, or to perform certain acts, since the bleeding into the pericardium leaks out of the wounds in the parietal pericardium into the pleural cavity and prevents tamponade of the heart.

Was the wound made before or after death?

Herzog (sec. 295, p. 243) states

A physician may be asked whether from the appearance of the wound itself he can tell whether it has been produced during life or after death. This is hardly possible with absolute certainty if the shot has been fired very shortly after death. Whether a bullet is fired into a living body or into a dead body, the mechanical effects of the projectile will be the same. A physician may often tell from surrounding circumstances, the spattering or squirting of arterial blood on the walls or from the position in which the body was found, that the bullet was fired into a living person. In case it was fired into a dead person a few seconds after death, there will still be some bleeding but there will be no arterial squirting. This, however, may often not be noticeable even if it has occurred, as the clothing, through which the bullet has passed may absorb the blood.

Furthermore, the necropsy will usually reveal whether the bullet has been the cause of the death, not only by determining

which vital organ has been perforated and the damage done, but also by excluding any other cause of death.

It has been the opinion of most authorities, therefore, that the main distinguishing point in wounds inflicted before and after death is the presence or absence of bleeding. It is true that cuts in the skin, when made after death, usually show no bleeding and contain no clots. Some bullet and stabwounds, however, when made soon after death, will bleed if they penetrate blood containing organs. For example, a wound of the heart made after death may cause a leakage of blood, by gravity alone, from the heart along bullet tract to the pleural cavity producing a hemothorax, and the blood in the pleural cavity may leak out a wound of exit and stain the surrounding clothes. There is no evidence, however, of arterial squirting.

I have seen a cisternal puncture, made twenty minutes after death, mimic exactly an antemortem wound, there being a blood clot sealing the puncture wound in back of neck and extensive underlying hemorrhage infiltrating the tissues along the needle tract.

In multiple bullet wounds of the body a careful autopsy usually discloses which one was fatal. There may be two, three or more fatal wounds all, or any one alone, producing death by hemorrhage and shock. On the other hand, insignificant wounds in the extremities or body walls that did not cause or influence death, may have, if the victim lived, become infected and prove fatal. This should be remembered when testifying as to fatal wounds.

PART II. DEATH FROM CUTTING AND STABBING

HOMICIDAL CUTTING AND STABBING

This has always been a common mode of murder. In this country it has been replaced largely by shooting which does not expose the murderer to a close fight with his victim.

Murder by cutting and stabbing is still, however, frequent among the American negro, being much more popular than shooting. Many of the victims show scars of a former encounter, usually with marked keloid formation.

In 360 homicides in Essex County during an eight year period (1925-1932) there were seventy deaths from cutting and stabbing, forming 19 percent of the total homicides.

In 2,457 homicides in New York City during a five year period (1928-1932) there were 499 murders by cutting and stabbing, or 20 percent of the total homicides.



FIG. 1. VIEWING BODY AT SCENE OF CRIME—HOMICIDE BY SHOOTING
"PUT ON SPOT"

Showing position of body when found. Shot twice in back of head and twice in left neck. Note two revolver wounds of entrance in left neck with powder marks beneath left ear. Shots at fairly close range (coup de grâce). (Office of Chief Medical Examiner, Essex County, N. J.)

At the present time, therefore, murder by cutting and stabbing forms about 20 percent of the homicides in and around the Metropolitan district and is only exceeded by shooting and assault. Furthermore, such homicides are seven times more frequent than suicide by cutting and stabbing. Therefore, every

case must be investigated with the same care as if murder was committed.

Cutting

In homicide by cutting the murderer most frequently attacks the throat, inflicting incised wounds or cuts. When the throat



FIG. 2. VIEWING BODY AT SCENE OF CRIME—HOMICIDE BY SHOOTING—
"TORCH MURDER"

Stolen seven passenger sedan used in taking gangster for "a ride." Victim shot in back of head and alcohol poured over body and set afire while still alive. Note charred remains of gangster. Autopsy required 18 hours. Police bulletin with accurate description of teeth, approximate age, height and weight of body, etc., mailed to over 30,000 dentists. Identified through teeth. (Office of Chief Medical Examiner, Essex County, N. J.)

is cut, the knife or razor is usually drawn clean across the front of the neck with considerable force, the murderer standing in back or to one side of the victim.

The edges are cleanly cut, straight, curved or zigzag, and

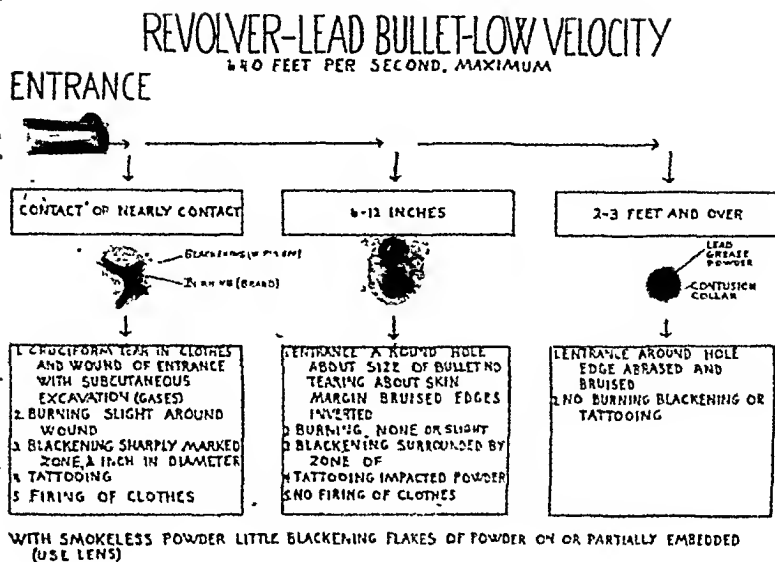


FIG. 3. DIAGRAM ILLUSTRATING WOUNDS OF ENTRANCE MADE BY REVOLVERS AT VARYING DISTANCES
(Office of Chief Medical Examiner, Essex County, N. J.)

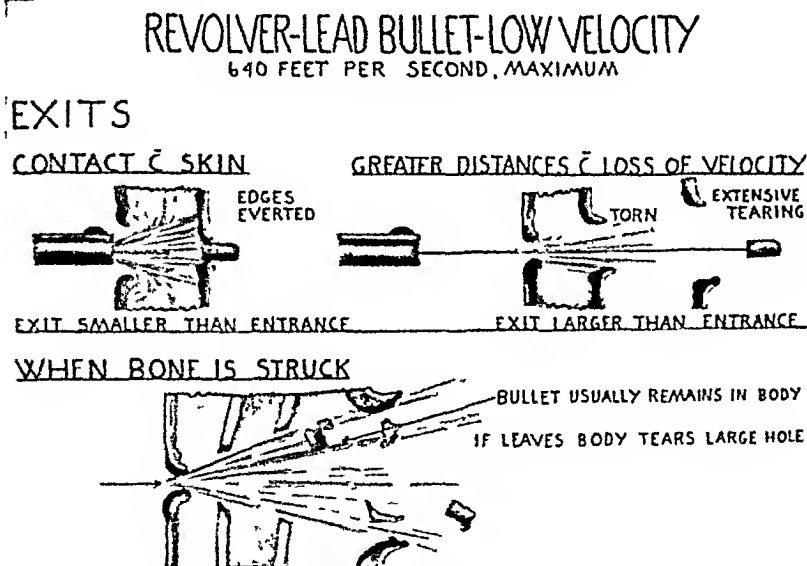


FIG. 4. DIAGRAM ILLUSTRATING REVOLVER WOUNDS OF ENTRANCE AND EXIT
(Office of Chief Medical Examiner, Essex County, N. J.)

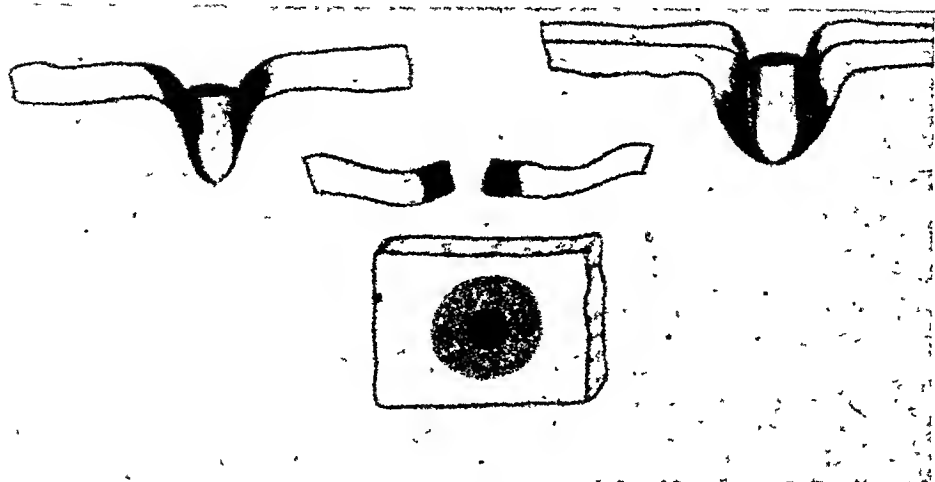


FIG. 5. SCHEMATIC REPRESENTATION OF THE PRODUCTION OF THE CONTUSION COLLAR IN WOUNDS OF ENTRANCE WHERE THE BULLET STRIKES THE SKIN AT RIGHT ANGLES

After Borri—Trattato di Medicina Legale. (Office of Chief Medical Examiner, Essex County, N. J.)

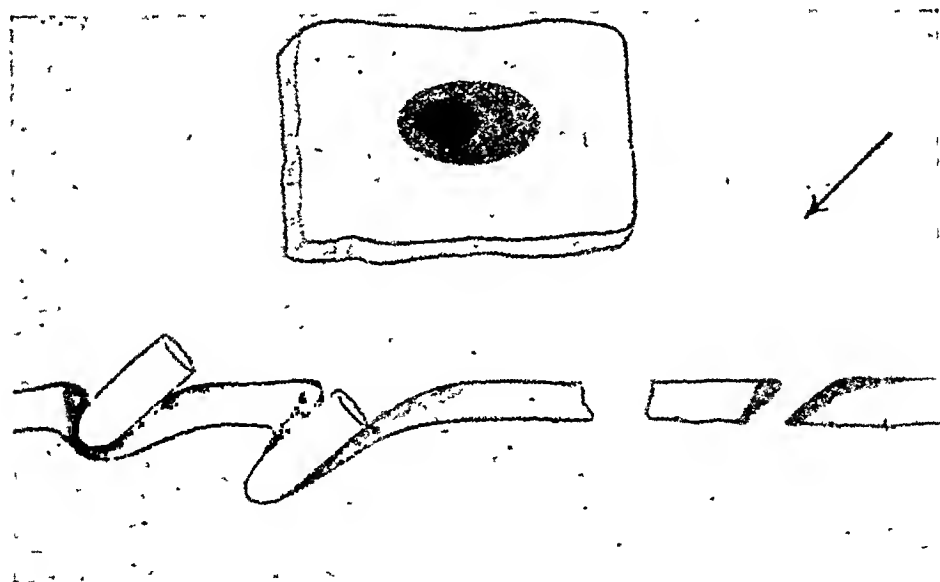


FIG. 6. SCHEMATIC REPRESENTATION OF THE PRODUCTION OF THE CONTUSION COLLAR IN WOUNDS OF ENTRANCE WHERE THE BULLET STRIKES THE SKIN AT AN OBLIQUE ANGLE

After Borri—Trattato di Medicina Legale. (Office of Chief Medical Examiner, Essex County, N. J.)

often everted. The wound gaps widest in its middle, especially if it cuts directly or obliquely across muscle tissue.

Most of the important structures of the neck may be severed, the knife often making a cut or scratch across the bodies of the cervical vertebrae or their intervertebral discs, and the head appearing as if almost decapitated.



FIG. 7. BULLET WOUND OF SKULL—SUICIDE

Deceased shot himself during pursuit by police after committing homicide. Note wound of entrance in right temporal region, a small punched out wound of outer table. This wound is beveled on inside. Note wound of exit over left parietal boss, a larger wound beveled externally. (Office of Chief Medical Examiner, New York City.)

One or both carotid arteries and internal jugular veins may be partially or completely severed, and evidence of arterial bleeding and squirting may be seen about the body and its surroundings. We have seen carotid squirting in a homicide, spray the ceiling of the room with innumerable droplets of blood. The ends of the severed carotid arteries are often found markedly retracted

in the wound. If the jugulars are cut, air bubbles are frequently found in blood of the right heart. In most of these cases death is practically instantaneous from hemorrhage.

On the side of the neck a homicidal cut may sometimes extend across the cervical spine and sever the well protected vertebral artery, a very difficult cut for a suicide.



FIG. 8. CONTACT AND NEAR CONTACT REVOLVER WOUNDS OF CHIN AND NECK—HOMICIDE BY SHOOTING

Note large lacerated wounds of entrance due to subcutaneous excavation by explosive gases. Most of powder smudge and burning, and tatooing is in the wounds themselves. The wound towards the midline, however, shows considerable tatooing of skin and is a near contact wound. (Office of Chief Medical Examiner—New York City.)

Often more than one cut is made. Cuts may cross each other and form large ragged flaps of skin and tissue, but there never will be found the delicate "hesitation marks" of a suicide.

Very often the neck and face will not be the only part of the body attacked: the trunk and extremities may show extensive cutting and stabbing. Frequently cuts may be seen over the fingers, hands, wrists and forearms, due to a struggle by the victim



FIG. 9. CONTACT REVOLVER WOUNDS OF FACE—HOMICIDE BY SHOOTING—
GANGSTER

Note two wounds of entrance larger than diameter of bullet with no surrounding powder burns or fatouing, and impression of muzzle and rib of pistol on skin. Muzzle and rib of pistol correspond with impressions on face shown on insert (Office of Chief Medical Examiner—New York City.)

in an attempt at defense. In an erotic murder we have seen as many as ninety-five separate cuts and stabwounds scattered over the entire body. There may be large cuts on the abdomen with partial evisceration.

In vendetta and gangster murders the murderers may finish



FIG. 10. CONTACT REVOLVER WOUND OF LEFT CHEST—SUICIDE BY SHOOTING

Note large wound of entrance with powder burn of skin and extensive laceration of heart with embedded black powder in heart muscle. (Office of Chief Medical Examiner—New York City.)



FIG. 11. CLOSE-UP REVOLVER WOUND OF FOREHEAD—HOMICIDE BY SHOOTING IN SPEAKEASY

Note close-up bullet wound of forehead with brand (flame burn) of skin eccentricly placed, tattooing, and shriveled burned hairs. The direction of the trigger of the gun was directly opposite the brand. Distance about 4". (Office of Chief Medical Examiner—New York City.)

the attack by drawing the knife across the face making a single or double cross, or cutting down into corner of the mouth—the so-called squealer's cut.



FIG. 12. MULTIPLE REVOLVER WOUNDS OF FACE AND NECK—HOMICIDE BY SHOOTING—GANGSTER

Note large contact wound on cheek with practically no surrounding powder burns or mark, and close-up wound on chin with extensive tattooing of surrounding skin, also close-up wound on neck with powder smudge and powder grains above line of collar. The surrounding skin over last wound is burned due to firing of clothes. (Office of Chief Medical Examiner—New York City.)

Stabbing

In stabbing the wound is a *punctured* one which penetrates the skin and enters the body to a variable extent. The wound of entrance on the skin is a narrow one, while the tract in the body tissues is usually a long one.

In stabbing, the blows are usually directed at the chest or

abdomen, rather than at the throat, with the idea of striking the heart or other vital organs. Stabwounds are often made from the back or side of the victim.

Insignificant stabwounds near the right or left border of the sternum frequently sever the internal mammary arteries producing hemothorax.



FIG. 13. HOMICIDE BY SHOOTING—GANGSTER TAKEN FOR "A RIDE"

Shot in back of head by gunman in rear seat. The position is typical of "a ride victim." The bleeding from the left ear is due to fracture of base of skull. (Office of Chief Medical Examiner, Essex County, N. J.)

Likewise apparently insignificant wounds made by penknives in Scarpa's triangle may sever the femoral artery.

Often repeated stabs may be made. These wounds may sometimes be delivered in rapid succession over a small limited section of the body. In an argument over a card game an old man re-



FIG. 14. BULLET WOUND OF ENTRANCE IN HOMICIDE SHOWN IN FIGURE 13

The blackening and burning of surrounding skin due to a close shot may be noted. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 15. HOMICIDE BY SHOOTING. WOUND OF NECK MADE BY BULLET FIRED FROM A DISTANCE

On the left--wound of entrance with contusion collar and absence of burning, blackening and tatooing may be noted. On the right--the larger torn wound of exit. (Office of Chief Medical Examiner--New York City.)

ceived nineteen stabwounds in his body. In another case thirty-two stabwounds were made apparently with an ice pick over a small area on the abdomen.

Stabwounds

If a sharp instrument such as a knife is used and the blow is perpendicular to the surface of the body, the skin wound is usu-



FIG. 16. HOMICIDE BY SHOOTING—BULLET SLAP OF ABDOMEN

To the right of the umbilicus is a tangential linear superficial brush abrasion of skin made by bullet which did not enter body. The fatal wound may be seen in the left upper chest. (Office of Chief Medical Examiner—New York City.)

ally a little larger than the widest part of the knife blade which has entered the body, as there is often a slight increase made in withdrawing the knife, or the blade may have entered at an oblique angle. Such wounds are usually elliptical in shape, gaping widest in their middle, with similar appearing ends in which the edges meet at acute angles. The wound is rarely wedge-shaped because of the elasticity of the skin. If, however, a knife

blade with a broad back penetrates a flat bone or cartilage, or solid viscus such as the liver, a wedge-shaped wound is sometimes seen.



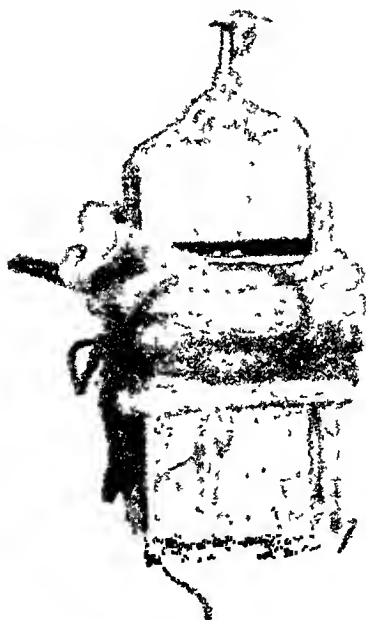
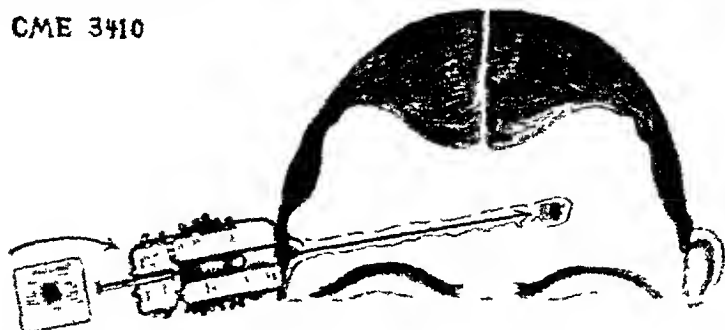
FIG. 17. SUBMACHINE GUN BULLET WOUNDS—GANGSTER "PUT ON SPOT"

Note multiple 45 caliber bullet wounds of head, body and extremities made at short range. Note extensive destruction of underlying tissues due to eccentric whirling of butt of bullets at this range, with fracture of right humerus. Also ploughing of tissues where bullets struck at angles. (Office of Chief Medical Examiner—New York City.)

Frequently in stabwounds there may be very little blood seen over the body or its surroundings, the bleeding being mainly internal. When examining the body at the scene of the crime

SUICIDE BY SHOOTING WITH MECHANICAL DEVICE

CME 3410

**FIG. 18. SUICIDE BY SHOOTING WITH A MECHANICAL DEVICE**

The suicide, a patient in an insane asylum, possessed a 38 caliber lead cartridge without the knowledge of the authorities. The patient, an ex-machinist, asked his brother, also a machinist, who was visiting him, to make the steel blocks. These metal blocks were supposedly to be used in the manufacture of wicker chairs in the occupational therapy department. The cartridge was put in the device which was then assembled with string and discharged. An ordinary nail was used as a firing pin. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 19. MATRICIDE BY SHOOTING WITH WINCHESTER 45-70 RIFLE AT A
DISTANCE OF ABOUT TWO FEET

The wound of entrance in left temple, with extensive burning and blackening, may be noted. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 20. SAME CASE AS FIGURE 19

The lacerated wound of exit with strand of tissue protruding externally may be noted. (Office of Chief Medical Examiner, Essex County, N. J.)

this may give a false impression that the murder has been committed elsewhere.

If the stabwounds are made with small instruments such as needles, an icepick, et cetera the wounds are usually round and the shape of the weapon, but frequently due to gaping of the



FIG. 21. HOMICIDE BY SHOOTING WITH 22 CALIBER RIFLE BY BOYS "POTTING" WINDOWS AT NIGHT

A bullet wound of entrance over region of left kidney causing death from internal hemorrhage may be noted. The rifle was fired at a distance of about 100 yards. The victim was in the bathroom near a window with the shade drawn, when bullet entered. Insert shows bullet hole in window. By methods of triangulation from the bullet wound in body, bullet hole in bathroom window, and bullet holes in neighboring houses, place of shooting was traced to an attic window 100 yards away. (Office of Chief Medical Examiner, Essex County, N. J.)

elastic skin, are a little larger in diameter than the weapon that made them.

If a butcher's steel or larger instrument is used, instead of a round wound there may be a linear stabwound, or an irregular or even triangular wound, frequently smaller in diameter than

the instrument producing it, due to contractility of the surrounding skin.

A careful examination of the edges of the surrounding skin should be made. Sometimes when knives are driven into the body with great force, a characteristic abrasion, pattern or laceration made by the hilt or other part of the knife, may be noted. This may help in the identification of a suspected weapon.

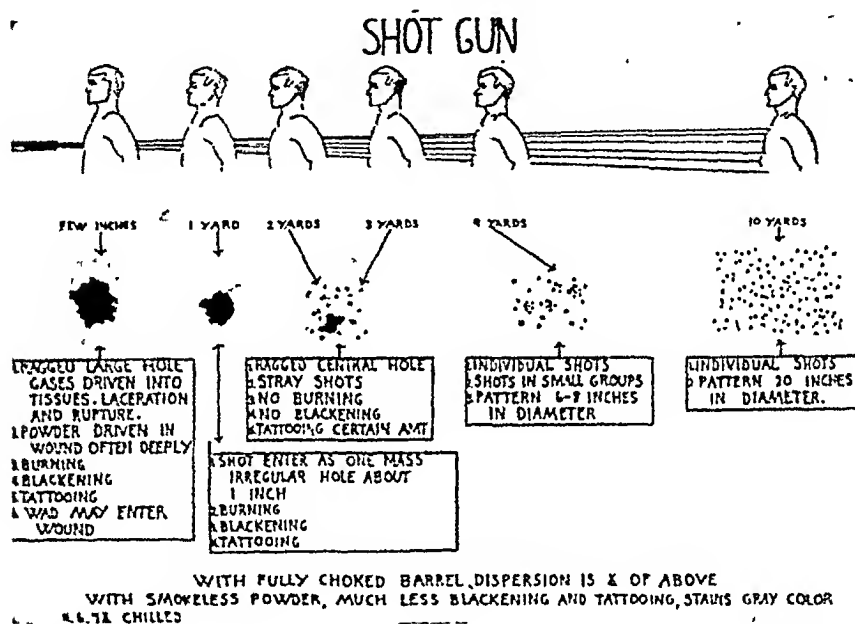


FIG. 22. DIAGRAM ILLUSTRATING WOUNDS PRODUCED BY A SHOTGUN AT VARYING DISTANCE

(Office of Chief Medical Examiner, Essex County, N. J.)

It will be seen that the greatest caution is necessary in forming an opinion or guess as to the character of the weapon used in stabbing. Only very general statements are safe and therefore, the signing of death certificates should be in a nonecommittal manner. They are official records and may be introduced in court. For instance, one may safely state: "Homicide by stabbing, multiple stabwounds of chest penetrating heart, made with sharp instrument."

Depth of stabwounds

Likewise the greatest caution must be taken in estimating the depth of a stabwound, unless the tip of the knife has stopped in some fixed part of the anatomy such as a bone. Under such circumstances the depth of the wound may equal the length of the weapon.

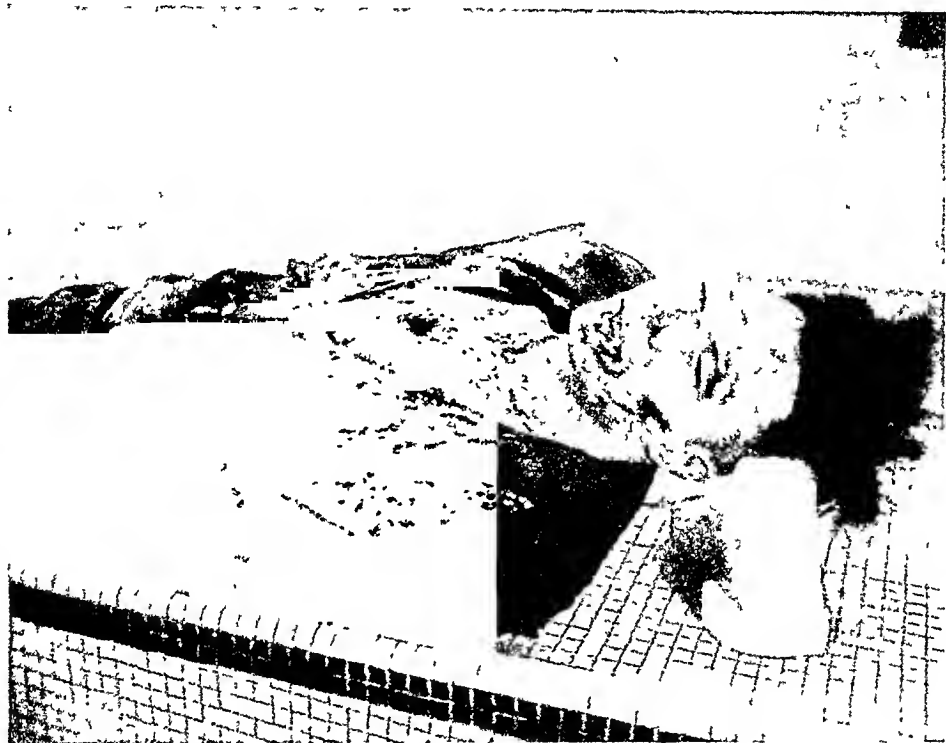


FIG. 23. HOMICIDE BY SHOOTING—SHOTGUN—STREET BRAWL

Note funnel-shaped wound of entrance in chest with scattering of small number of shot above wound. Range about four feet. Multiple lacerations of heart and kidneys. (Office of Chief Medical Examiner, Essex County, N. J.)

Frequently the estimated depth of the wound at necropsy may be greater than the length of the blade of the weapon. This is often seen in stabwounds of the heart when the heart is nearer to the anterior chest wall in the erect position of the body at the time of stabbing, than in the prone position of the body at necropsy. Furthermore, the force of the blow may depress soft parts and allow a short blade to go a greater distance, as is often seen in wounds of the abdomen.

At necropsy the same care should be taken as in bullet wounds to accurately describe the position of the wounds. They should be photographed and their course in the body and the structures injured should be described. The depth of a wound is measured from its point of entrance in the skin to the deepest part reached by the weapon, which is often in viscera which have a considerable



FIG. 24. HOMICIDE BY SHOOTING—SHOTGUN

While walking along street shot in back by insane person, distance of about 30 feet. Over 370 No. 8 bird shot entered body, many penetrating lungs and abdomen. Note pattern about 20 inches square over back of body. (Office of Chief Medical Examiner, Essex County, N. J.)

normal mobility in the body. Then maximum and minimum estimates of the probable depth of the wound should be made.

Search should always be made in the wounds, especially where bone is struck, for broken pieces of the weapon. They may fit exactly into a suspected weapon. However, such occurrences are rare.

Weapons used. In judging the nature of the weapon used it

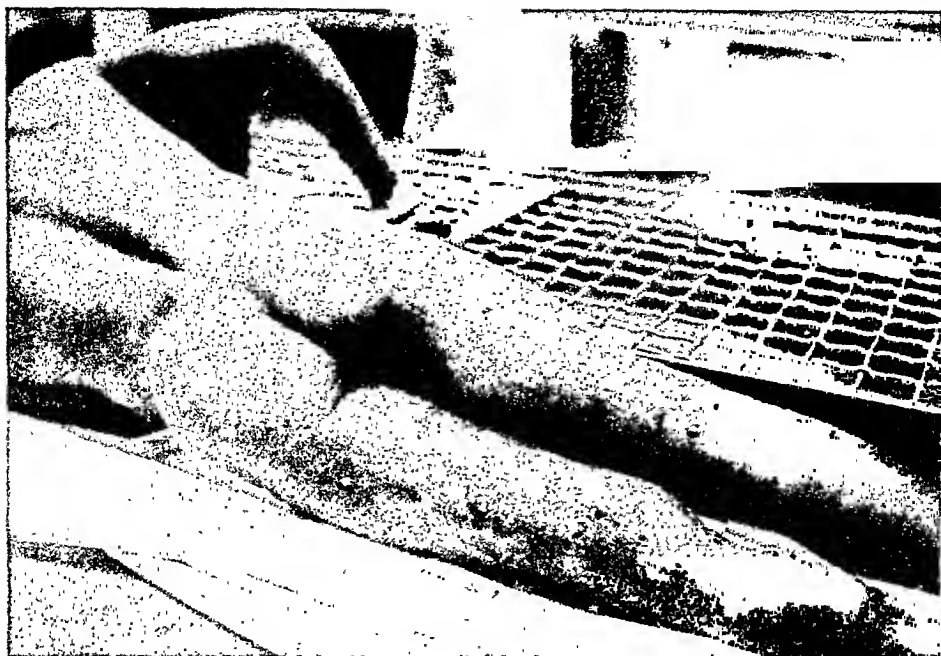


FIG. 25. HOMICIDE BY SHOOTING—SHOTGUN

Shot with shotgun in drunken brawl. Over 200 No. 7 bird shot entered back of both knees lacerating both popliteal arteries and veins. Died from hemorrhage on arrival at hospital. Intoxicated. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 26. HOMICIDE BY SHOOTING—SHOTGUN

Note nine wounds of entrance made by "00" slugs. (Office of Chief Medical Examiner—New York City.)

should be borne in mind that a hatchet, cleaver or razor will make incised wounds, but a razor will not cut into bone. A blow on the head with a pipe or sharp board may split the scalp and resemble a cut, but on careful examination often brownish, parchment-like linear brush abrasions may be noted along the margins of the wound. Bullets may make tangential splits in the skin that appear like cuts.

Homicidal assaults

Space does not permit a discussion of wounds produced by such weapons as hatchets, axes, hammers, et cetera, which often cause incised or punctured wounds. The great weight of these weapons and the violence with which they are used usually inflict wounds about which there is little argument.

Among the Chinese in their tong wars, hatchet murders are considered the orthodox style of killing. The murderer is usually paid a higher fee than for shooting or stabbing his victim.

The presence of blood or hair of the victim on suspected weapons is the work of the toxicologist or crime detection laboratory.

SUICIDE BY CUTTING AND STABBING

Suicide by cutting and stabbing is on the decrease, being greatly exceeded by inhalation of illuminant gas, hanging and jumping from buildings.

In 1,056 suicides in Essex County during an eight year period (1925-1932) there were only thirty-eight by this method, forming but 3 percent of the total cases.

In 7,219 suicides in New York City during a five year period (1928-1932) 241 were by cutting and stabbing, forming also only 3 percent of the total suicides.

Suicides usually cut themselves, stabbing being much more uncommon. The most frequent locations are the throat and wrists, often a combination of both. The weapon used in cutting is usually a sharp knife, butcher knife, razor or safety razor blade. If the latter superficial cuts on the hand using the instrument may be sometimes noted.

A right handed man in cutting his throat usually starts the cut high up on the left side of his neck below the angle of the jaw. Frequently, but not always, he will make one or more, sometimes five or six, superficial scratches or deeper cuts before letting the knife go across the front of his neck. These are typical "hesitation marks" characteristic of suicide. The cut usually extends across the front of the neck slanting downwards from left to right, or in a horizontal manner. If the cut is deep enough the carotid vessels on the left side of the neck may be severed. The cut then usually passes across the top of the thyroid notch or lower, crossing the anterior triangle of the neck on right side, often severing the carotid vessels on this side. It usually stops near the anterior border of the sternomastoid muscle where two to four deep serrations may sometimes be seen. Left handed suicides will usually cut in the opposite direction.

It must be remembered that in ambidextrous people, a man thought to be right handed (because he writes with his right hand) may use his left hand in cutting his throat.

One or both wrists may be cut, the cuts practically always being situated over their anterior surfaces about one to two inches above the wristjoint. There may be only a few, one to three, superficial or deep cuts which sever radial arteries. More often there are many parallel superficial scratches and cuts (hesitation cuts), sometimes fifteen to twenty or more. Curiously these hesitation cuts often do little harm and he is forced to finish the job by other means.

In some cases multiple attempts are made. In one case the victim stabbed himself repeatedly in the scalp and then cut his throat. Failing at this he hung himself with a leather belt. The belt broke and his body in falling knocked off a gas fixture causing death by asphyxiation.

Weird and unusual stabbing methods sometimes occur. In one case a despondent patient stabbed himself through a craniotomy wound. Of course, in all suicidal stabbing cases the location and direction of the wound must be compatible with suicide.

ACCIDENTAL CUTTING AND STABBING

Accidental cutting causing death is sometimes seen. Children may cut themselves while playing with fragments of broken glass, pottery, et cetera. I had one case of a child who while running with a bottle of milk, fell and drove a large sharp piece of glass into his abdomen producing internal hemorrhage.

In another case a child was accidentally stabbed through the perineum by the stalk of an old umbrella placed by other children at the bottom of a playground slide.

In adults, fencing accidents have occasionally resulted in death. In one of my cases the foil entered the orbit and penetrated the brain. The two fencers had removed their masks and were just demonstrating a new thrust.

In industry, various accidental cuttings may cause death by injuries to body and extremities. These have been greatly reduced, however, by the activities of safety councils, and other agencies. In one case a long splinter of wood flying from a buzz saw produced a stabwound of the abdomen causing death.

PART III. VIOLENT DEATH FROM ASPHYXIA

Asphyxia is the result of interference with the respiratory function whereby the vital organs and tissues are prevented from obtaining the supply of oxygen essential to life.

Asphyxia may be due to either mechanical causes preventing the entrance of oxygen into the lungs; to injuries and diseases interfering with the movements of respiration; to the presence of irrespirable gases, or to causes acting upon the respiratory centers such as alcohol and certain poisons.

GENERAL PICTURE OF ASPHYXIATION AT NECROPSY

External examination

The body usually shows a face which is cyanosed. The lips, fingernails and ears are bluish in color and may be almost black. The eyes bulge and the conjunctivae are reddened and suffused. Petechial hemorrhages may be seen in the skin, especially over the face, forehead, neck, and commonly in the palpebral and ocular conjunctivae.



FIG. 27. HOMICIDE BY CUTTING THROAT

Note clean cut made by knife drawn across throat of victim. Also note absence of hesitation marks as seen in suicidal cut throats. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 28. HOMICIDE BY CUTTING

Old lady murdered by maniac. Multiple slashes of throat severing carotid vessels. (Office of Chief Medical Examiner, Essex County, N. J.)

The tongue more frequently lies behind the teeth, or if protruded, is caught between the firmly locked teeth. Postmortem lividity over the dependent parts of the body is marked, and due to venous distention the picture of cyanosis predominates. However, in some cases there may be pallor instead of cyanosis.

The cooling of the body may be delayed. Rigor mortis usually sets in early as it does in most sudden deaths.



FIG. 29. HOMICIDE BY CUTTING AND STABBING--EVisCERATION

Murdered by maniac. (Office of Chief Medical Examiner, Essex County, N. J.)

Internal examination

The right heart is overdistended with fluid blood which is a very dark color (except in carbon monoxide poisoning) and contains no clots. The left heart is contracted and empty. The large veins of the superior mediastinum are enormously distended with dark fluid blood.

The lungs usually well fill the pleural cavities, their anterior



FIG. 30. EROTIC MURDER—STABBING AND CUTTING—SCENE OF CRIME

Body of Indian girl lying in hallway of tenement house. Multiple stab-wounds of chest and throat causing death from hemorrhage. (Office of Chief Medical Examiner, Essex County, N. J.)

borders often meet or overlap in the midline opposite the third rib. Blebs of acute interstitial emphysema may be noted over the external surfaces of the lungs, especially over their anterior

edges and external margins at their bases, where often rows of minute blebs occur. In some places actual rupture of these vesi-



FIG. 31. BODY OF VICTIM SHOWN IN FIGURE 30

Body shows over 95 stabwounds and cuts, most of them made after death. Note absence of bleeding in most of the cuts. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 32. THE SAME BODY SHOWN IN FIGURE 31
(Office of Chief Medical Examiner, Essex County, N. J.)

cles occurs with a resultant suffusion of air beneath the pleura, probably due to spasmodic expiratory efforts. On section, the lungs are often bloody and markedly congested. The bronchial



FIG. 33. HOMICIDE BY CUTTING AND STABBING

Stabwound of right upper chest severing internal mammary artery, hemothorax. Superficial cut across right arm. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 34. HOMICIDE BY STABBING WITH PEN-KNIFE

[Note small incised wound in lower part of Scarpa's triangle on right side. Severance of femoral artery. Dead on arrival at hospital. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 35. HOMICIDE BY STABBING AND CUTTING

Note two stabwounds of chest penetrating heart, and superficial cut across outer aspect of left wrist received in struggle. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 36. INCISED WOUND OF FACE AND NECK - HOMICIDE

Note large cut across left side of face and neck made by murderer. The posterior end of the cut is deep, leading down to the vertebra with severance of the well protected vertebral artery. Death from hemorrhage. (Office of Chief Medical Examiner—New York City.)



FIG. 37. MULTIPLE STABWOUNDS—HOMICIDE

Note multiple small stabwounds of chest (32 wounds) penetrating heart, such as might be made with ice-pick, file, stiletto, or similar instrument. Body found submerged in bath tub. (Office of Chief Medical Examiner—New York City.)



FIG. 38. WOUNDS BY STABBING AND CUTTING—HOMICIDE

Note multiple stabwounds of chest, disembowelment and a double cross vendetta wound on face, the lower cut simulating the so-called "squealer's cut." (Office of Chief Medical Examiner—New York City.)



FIG. 39. WOUNDS MADE WITH AXE—HOMICIDE

Note severance of left side of neck, chopped mandible and left index finger. At autopsy 18 hours after death, right auricle was found beating. (Office of Chief Medical Examiner—New York City.)



FIG. 40. HOMICIDE BY ASSAULT—AXE MURDER

Head recovered from river about two weeks after murder. While intoxicated, struck in back of head with axe fracturing skull. Body dis-membered with saw and axe into nine parts, eight of which were recovered, some several miles apart. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 41. SUICIDE BY CUTTING THROAT WITH RAZOR

The cut in this case started on left side of neck and was finished on right side, being made with the right hand. Note numerous chops and "hesitation cuts," characteristic of suicide. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 42. SAME CASE AS IN FIGURE 41

Showing right side of neck where cut ended. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 43. SUICIDE BY CUTTING THROAT AND WRISTS WITH RAZOR

The "hesitation cuts" on left side of neck where cut was started may be noted. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 44. SAME CASE AS IN FIGURE 43

Showing multiple razor cuts on both wrists with an occasional hesitation mark. (Office of Chief Medical Examiner, Essex County, N. J.)

mucosa may be intensely congested. An acute edema is sometimes noted, microscopic examination showing rupture of the interalveolar septa and distention of the alveoli with fluid which contains neither leucocytes or fibrin.



FIG. 45. WOUNDS BY STABBING AND CUTTING—MULTIPLE ATTEMPTS AT SUICIDE

This man cut his throat and stabbed himself numerous times over head, using his right hand. Being unsuccessful in severing any large artery, he then hanged himself with a leather belt which broke. While falling he knocked off the tip of a gas fixture and was asphyxiated by illuminant gas. (Office of Chief Medical Examiner—New York City.)

Subendothelial ecchymoses, varying in size from 1 mm. to 1 cm. in diameter, may be found over the parietal and visceral pleura, the mediastinal pleura, the visceral and sometimes the parietal pericardium, the endocardium, the meninges and more rarely the peritoneum. These are valuable signs of asphyxia but must be distinguished from similar appearing hemorrhages occurring

in sepsis, purpura, nephritis and some of the poisons, notably arsenic and phosphorus. In cases of slow or very rapid asphyxiation no ecchymoses may be found. They are almost constantly found in overlaying.



FIG. 46. SUICIDE BY STABBING

Note stabwound over left parietal region made by left handed person in committing suicide. The stabwound passes through an old craniotomy opening directly into brain, the scar of healed skin flap being visible. (Office of Chief Medical Examiner--New York City.)

Asphyxial hemorrhages in the scalp are often seen in hanging and strangulation cases. The brain and pia-arachnoid is usually engorged with blood, the veins being prominent and distended. The grey matter is often a purplish hue. Occasionally asphyxial hemorrhages may be noted in the meninges and parenchyma of the brain.

The abdominal viscera, especially the liver, spleen and kidneys

are dark red in color and filled with blood. The gastrointestinal tract is often cyanosed.

In some cases of sudden occlusion of the larynx by a foreign body, such as a piece of meat, laryngeal tumor, or in some individuals who fall or are thrown into cold water, death may occur very suddenly, apparently by reflex paralysis of respiration. Again, in some cases of hanging or strangulation sudden paralysis of respiration and heart action may take place due to compression of the vagi. In such cases, there may be absence of cyanosis, excessive hyperemia and ecchymoses, making the cause of death, at times, a difficult one to determine.

When most of the above picture is present, it is typical enough to form the opinion that death was the result of asphyxia. When only a few of the signs are present the correct interpretation is often difficult.

Whether the asphyxia was due to violence or only a mode of death during the course of a disease cannot be stated unless evidence of the asphyxiating cause is found, such as, water in the lungs in drowning, mud in the nose and mouth in smothering, a piece of meat in the larynx in choking or the presence of a high concentration of carbon monoxide in the blood in illuminant gas poisoning.

ASPHYXIA DUE TO MECHANICAL CAUSES

In asphyxia due to mechanical obstruction breathing is prevented, the tissues in consequence are depleted of oxygen (anoxemia) and retain an excess of carbon dioxide.

Smothering

Smothering is defined as being a closure of the external respiratory orifices, namely, the nose and the mouth, produced either by the hands or by other means.

From the viewpoint of the medical examiner the more frequent types of smothering occur during infancy.

Overlaying. A baby may be overlaid by the mother either accidentally or with homicidal intent. Overlaying is common amongst the poor where the parents and the infant sleep in the

same bed, especially when the parents are intoxicated. In such cases, the body may show little or no evidence of external injuries; however, there may be a slight or marked cyanosis especially of the head, neck, finger nails, et cetera, or the presence of bloody mucus in the nostrils and mouth.

Postural smothering. On the other hand, a baby may be accidentally smothered by the pillow or bed clothes, often in the crib, by turning over in sleep or during an unexplained convulsion.

Infanticide. In a similar manner, homicidal smothering may be accomplished by holding or pressing a soft pillow or bed clothes over the baby's face leaving practically no external marks of injury.

The external examination of the body in all these cases will be practically the same, there may be no external marks of injury or merely a flattening of the nose and cheeks as a result of pressure. The pallor over the compressed parts accentuate the surrounding lividity. Bloody mucus or froth may be found in the nostrils or mouth, often staining the underlying bed clothes.

The necropsy shows the general picture of death from asphyxia, sometimes pronounced and at other times less definite. In smothering, more than in any other type of asphyxial death, small asphyxial petechial hemorrhages may almost invariably be found over the parietal and visceral pleura, the visceral pericardium or in the mediastina, tissues, meninges or peritoneum.

Accidental smothering in adults. In healthy adults accidental smothering by bed clothes or postural smothering by turning the head into the pillow is practically impossible as the person, by reflex action, wakes up and throws off the obstructing clothes. It may occur, however, in acute alcoholism, status epilepticus or in debilitated or dying persons.

Suicidal smotherings. For similar reasons suicidal smothering is rare since the individual can seldom hold anything over his mouth or nose long enough. On the other hand, self-strangulation by means of a ligature is not uncommon, since the compressing ligature produces rapid loss of consciousness. I had one case of an old lady who, by holding a wad of cotton containing a

little chloroform over her face, smothered herself, death being due to asphyxiation.

Homicidal smothering. Smothering by means of cloths, or other materials, is unusual except in infanticide or in weak persons. In healthy adults, a struggle usually taking place, the murderer has to resort to some other means. External injuries received in the struggle are therefore apt to be found.

Homicidal smothering by means of applying the hands to the face and mouth is more common and is known as "burking." This method of murder originated during the "resurrection days" when anatomists, to obtain cadavers, were compelled to deal with grave-robbers. In 1827, Burke and his partner Hare supplied bodies for the famous Edinburgh anatomist, Robert Knox, by murdering unfortunates in this manner. They usually threw their victims to the ground and knelt on their chests with the whole weight of the body while one hand was clapped over both the nose and the mouth, the other held the jaws firmly pressed together. Death usually took place in three to four minutes, leaving very few external marks of injury. To one untrained in this technic, however, more or less typical marks of injury may be seen around the nostrils, lips, and other exposed parts.

Other methods. Finally, smothering may take place, usually accidentally, by falling into mud, sand, ashes, water, et cetera. I examined the body of a man who by falling into a large hopper filled with fine wood shavings, was smothered; the entire upper respiratory tract as far down as the finer bronchi contained wood shavings. In another case there five men were overcome by arseniuretted hydrogen while cleaning a waste pit, death was due to smothering by the mud at the bottom of the pit.

STRANGULATION

Asphyxia by strangulation is due to a closure of the air passages by external pressure on the neck.

Three main types of strangulation may be described:

1. Hanging.
2. Strangulation by ligature.
3. Throttling or manual strangulation.

Hanging

In hanging, the body is completely or partially suspended by means of a rope, wire, belt, strap, sheet, et cetera, placed around and constricting the neck by weight of the hanging body.

Suicide, murder or accidental? Hanging is almost always suicidal. It ranks next to illuminating gas as a means of committing suicide.

In New York City during a five year period (1928-1932) there were 1,044 suicides by hanging, only six due to accident and none due to homicide. Hanging formed 14 percent of the total 7,219 suicides.

This should not cause the medical examiner to quickly and carelessly dispose of all hanging cases as suicidal without a careful investigation. Murder by hanging must be very rare since it requires at least the help of another person unless the victim has been previously rendered unconscious by means of other injuries, drugs or poisons.

Suspension of the body to simulate suicide after murder has been committed, is more common than murder by hanging. The investigation and necropsy must show that the hanging occurred postmortem and that some other violence or poison had produced death.

Smith states that the presence of ecchymoses about the mark of the ligature, a red line of rupture of the intima of the carotid arteries and the presence of definite ecchymoses or areas of extravasated blood are the only reliable signs that the body was suspended during life. A colored zone at the edges of the furrow may be due to postmortem hypostasis and is not reliable. Practically all the other signs of hanging have been produced experimentally by Smith. The depression of the tissues by the ligature is much the same whether the body is suspended before or after death.

Of course the examination at the scene of the death may give clues of great value. For instance, in a case reported by Smith in which the victim had first been strangled and the body later suspended, although the feet were two feet above the floor, there was neither table nor chair from which he could have stepped.

Accidental hanging is unusual and in our experience has occurred chiefly in children who were playing cowboy, bandit, and other such games. The history and examination of the scene of death usually clears the case. Some of the children have undoubtedly committed suicide however, but have been given the benefit of the doubt. Occasionally a child may be accidentally suspended by a neck band of clothing from a projecting railing or by falling, catch his neck between the palings of a fence and hang.

Suicidal hanging. By far the commonest way of committing suicide by hanging is to stand on a chair or box, adjust the loop or running noose about the neck and kick the support over.

The downward pull of the body causes the rope to be pulled upwards to the highest position possible on the neck where it is usually stopped by the angles of the jaw. In front, the rope lies between the thyroid cartilage and the hyoid bone, on the sides just under the angle of the lower jaw and below the ears and behind, runs across the scalp where it reaches the highest point.

In such cases, where there has been no appreciable drop and therefore no injury to the spine and medulla, the general constriction of the neck causes partial or complete compression of the great vessels of the neck, interfering with the cerebral blood supply and causing rapid unconsciousness. The entrance of air into the trachea is prevented by the closure of the glottis, by the upward and backwards displacement of the tongue, soft palate, and other structures, with the production of asphyxia. Compression of the vagus nerves may cause cardiac inhibition.

The rapidity with which consciousness is lost is sometimes possible to evaluate by noting the hand of the victim to be still in the noose or one foot still on the chair or stool.

To account for death in these hangings where no "drop" occurs, pressure on the blood vessels of the neck, pressure on the nerves, and mechanical obstruction to breathing have been proposed, separate and exclusive claims having been made for each. In still other contributions it is admitted that two or all three of these major effects may aid in causing death, and contentions have arisen over the significance to be assigned to each.

It is generally accepted that the soft palate, tongue and larynx

are pushed up and back so as to cut off the respiration effectively when junction of the body and its suspensor are in the typical position on one side behind or otherwise close to one ear. Access to air, it has been shown, has not been obstructed in persons who have hung themselves after tracheotomy, the tubes still being in place below the loop. In some of the ancient forms of execution, the trachea was cut or stabbed before the criminal was suspended by the neck.

Observations have been made of a short stoppage of the heart due to irritation of the vagi at the moment of suspension; the inability to resuscitate persons cut down almost at once has been accounted for in this way. Others have called attention to the superficial location of the superior laryngeal nerves and their important connections with cardiac ganglions and nerve plexuses. In these hangings death usually results in from five to ten minutes after consciousness is lost, even though the heart may continue to beat for eight or ten minutes after the cessation of respiration. In view of this latter fact resuscitation is possible, and artificial respiration should be started not later than five or six minutes after suspension.

From the above it will be seen that most authorities consider asphyxia by hanging as due to a combination of these three major factors, the death being not one of pure asphyxiation. I am somewhat skeptical about this. My experience has been that the main factor in these hangings is asphyxiation due to closure or shutting off of the air passages and that all other factors are usually of secondary or contributory importance.

That this is not more clearly recognized is due to the fact that the anatomical relationship of the structures in the mouth, nasopharynx, and glottis cannot be demonstrated by the ordinary necropsy technic.

In the cases of suicidal hanging where I have taken the trouble to split the head in a vertical transverse manner, (after the brain has been removed, by sawing through the middle of the basilar portion of the occipital bone and exposing the nasopharynx from the rear or by making median sections of the entire head and neck) I have repeatedly demonstrated complete obstruction to the air passages.

In suicidal hanging it is not necessary that the body be completely suspended. Suicides often hang themselves in a sitting or reclining position with the feet and even the entire body on the floor, only a comparatively slight traction being required to produce asphyxiation. Usually in these cases the mark of the ligature is lower down than when the body is suspended, often below the thyroid cartilage and the furrow sometimes runs almost horizontally around the neck.

Necropsy. In suicidal, accidental and homicidal hangings, in which there is no "drop," the following may be found at necropsy:

Furrow or constriction mark. This is by far the most important and often the only absolute sign of hanging.

If the body is completely suspended the lowest part of the furrow is usually in front, above the thyroid cartilage, and its course will depend on the position of the knot. A convergence of the furrow behind one or the other ear, under the angle of the jaw on either side, under the chin, or under the occiput denotes the position of the knot which is usually the highest part of the furrow.

When a fixed loop is used the head is tilted to the side opposite the open end of the loop, the furrow being deepest and most marked on the side opposite the knot, and gradually inclining upwards in an oblique manner towards the knot. Marks produced by the knot are often absent, since if the loop is a loose one there will be no pressure of the knot against the skin. There may be only the converging ends of the furrow with an area of unmarked skin separating them.

When a running noose is used the same position of the head results, the furrow, however, is usually less oblique and the marks of the knot are often seen since it is pressed tightly into the skin.

If the body is only partly suspended the course of the furrow is, as a rule, more circular and in front lies lower often below the thyroid cartilage.

If the ligature is a rope or of hard material, a single or double furrow is seen whose depth is pale, and whose margins are congested, hyperemic and occasionally show a few ecchymoses especially on its upper border, proving that suspension took place

during life. On drying the furrow often becomes brown in color and parchment-like.

If the ligature is soft, such as a sheet or scarf, one may find only a broad pale depression which may disappear in time and leave practically no mark.

If a belt or suspenders were used, the marks of the buckles may sometimes be recognized.

External examination of body. The face above the furrow is often pale, however, if the strangulation has been slow and not too tight, resulting in first the compression of the veins before the deeper arteries, marked cyanosis may be present above the ligature.

The position of the tongue behind or in front of the teeth in the mouth depends entirely on the relation of the ligature to the hyoid bone and the direction of the traction.

Saliva often dribbles from the mouth when the head is tilted forward due to pressure of the ligature on the salivary glands.

If the body has hung for some time, the blood settles into the lower extremities and the most marked lividity may be seen below the waist, the upper part of the trunk being pale. Even petechial hemorrhages may occur in the legs in this manner.

It is a common belief that an erection of the penis with discharge of semen occurs in hanging. Gravity alone will account for the engorgement of blood in the penis and the leaking of fluid from the seminal vesicles.

Internal examination of body. Just as the external marks of constriction on the neck form the main diagnostic signs of hanging, so the effects of this constriction on the neck form the most important findings at necropsy. The other findings only establish that death was or was not due to asphyxia without determining its cause.

I have found in most cases of hanging that the direction of the constricting force which is almost always upwards and backwards pulls the hyoid bone with the base of the tongue backwards and upwards. The normal space between the base of the tongue and epiglottis is obliterated so that the base of the tongue lies in apposition with the anterior surface of the epiglottis. The epi-

glottis is pushed backward so that its posterior surface is in apposition with the posterior pharyngeal wall effectively blocking the glottis. The soft palate and uvula is pushed backwards and upwards, its posterior or superior surface coming in apposition with the posterior pharyngeal wall effectively blocking the passages of air from the nasal cavities.

As death has occurred with the parts in these positions they may be found months after burial provided the body is fairly well preserved.

The position assumed by the tip of the tongue usually depends on the relation of the ligature to the hyoid bone and the direction of the traction. If the ligature comes just below the hyoid the tongue is forced upwards and slightly backwards, its tip being forced forwards so that it may either protrude through the lips, be caught between the teeth or, if the jaws are firmly closed, behind them. If, however, the ligature is very high and the main pressure is above the hyoid, the base of the tongue is forced upwards and backwards and the tip will be found behind the teeth pulled well back into the mouth. This same position occurs when the ligature is very low and the traction is more circular.

In addition to the position of the parts described above, there may be found in the dissection of the neck, evidence of contusion, laceration and hemorrhage in the tissues underlying the area of compression.

The tissues directly under the ligature may be dry, pale, compressed or show ecchymoses. Fibers of the platysma may be torn. The trachea may be compressed and some of its rings broken, the thyroid cartilage may be fractured, especially its posterior horns. The hyoid bone which is often fractured in high manual strangulation escapes injury in hanging.

Laceration of the intima of the carotid arteries at the site of compression is sometimes seen producing a transverse tear. The mucosa of the trachea, epiglottis and nasopharynx may show ecchymotic hemorrhages and be markedly hyperemic and bloody mucus be found in the nasopharynx.

Often many of these signs are missing or present to only a slight degree, especially when hanging occurs by means of softer liga-

tures. The position of the parts in the throat, however, will almost always be found. Occasionally, but not often, in hanging with the loop of knot placed behind the ear, and with sudden tension of the body weight upon the noose, fracture or dislocation of the atlas upon the axis, with crushing of the medulla by the odontoid process, occurs, and death is instantaneous. The findings above described in such cases may be absent. In addition the necropsy usually shows the general findings of asphyxiation none of which alone are characteristic.

Judicial hanging. In many of the States hanging is the official method of execution and when performed properly by using the "drop" and allowing the body to fall some two or more feet, the sudden jerk on reaching end of rope causes dislocation or fractures of the spine and rupture of the cord. Death is instantaneous and is not due to asphyxia unless the hanging is poorly done. The heart may beat for several minutes and muscular convulsions may set in after a few minutes of quiet.

In lynching by hanging, the rope is usually placed around the neck and the other end thrown over a tree or pole, the victim is then pulled up until his feet are several feet above ground. Death usually occurs by slow asphyxiation with marked cyanosis of face.

Strangulation by ligature

Strangulation means a constriction of the neck with a ligature, the force being applied directly to the ligature. The mark encircles the neck, and is lower and more horizontal than in hanging. The ligature presses directly upon the larynx and asphyxia almost alone results.

Murder, suicide or accidental? Strangulation is a fairly common form of murder. Often the murderer uses much force and injuries to deep structures may be found together with other evidence of violence. The tearing of clothes and surrounding evidence of a struggle is often found. The ligature may be found around the neck or it may have been removed. If present it should be photographed before removal and the knot should never be untied as it may form the most important evidence

against the murderer. The ligature can be readily cut off while still preserving the knot.

In New York City in a five year period (1928-1932) there were thirty homicides by strangulation with a ligature, only four suicides and no accidental strangulations.

Suicide by strangulation is usually accomplished by tying a handkerchief, shoe-string, cord, wire, necktie, belt, or suspenders around the neck. As a rule several turns are made and then a knot is tied. In spite of the fact that consciousness is lost very rapidly, the suicide ties a firm knot. Or, he may use a stick of wood, spoon or other object and turn a loose noose around, in tourniquet fashion, which is not able to untwist after he falls, the compression still being maintained.

In suicide the ligature should be found on the neck and no signs of other violence or marks of a struggle should be seen.

Prisoners often commit suicides by hanging themselves in their cells by means of belts, suspenders or neckties so that in well regulated prisons these articles are taken away or do not form a part of the prison uniform.

Accidental strangulation is only occasionally seen, occurring especially in children in play who may be caught in window sashes, cords or ropes. The surrounding circumstances and history is usually plain.

Necropsy. As the ligature is not so suddenly applied as in hanging, the large veins of the neck are compressed before the arteries and the face is more frequently cyanotic, often blue or black, swollen, eyes bulging, asphyxial petechial hemorrhages in the conjunctiva, in the skin of the face, in the scalp, protrusion of tongue with indentations from teeth and blood stained mucus in the mouth and nostrils.

In murder where greater constriction usually occurs extensive laceration and hemorrhage in the deeper tissues may be found. Fracture of the larynx and rings of the trachea may be seen. The general picture of an asphyxial death is usually more marked than in other forms of asphyxiation. The petechial asphyxial hemorrhages in the pleura, pericardium, and blebs of acute interstitial emphysema in the lungs are almost always present and

usually pronounced. All the important organs are engorged with dark fluid blood. Putrefaction takes place rapidly often confusing the picture. The best preserved tissues will be found directly under the compressed area where definite hemorrhage may still be seen in such cases.

Furthermore, if a very soft cloth has been used, external marks on the neck and laceration and hemorrhage in the tissues of the neck may be absent or slight in extent.

Throttling or manual strangulation

Manual strangulation is always homicidal. It would be difficult to conceive of a suicidal or accidental throttling by means of the hands.

In New York City, in a twelve year period (1918-1930) in a total of 3,340 homicides investigated by the Chief Medical Examiner's office, Gonzales⁴ reported twenty-four cases of manual strangulation or 0.7 percent of the total homicides, indicating the comparative rarity of this form of violent death. Nineteen, occurred in adults, from twenty-three to sixty-eight years of age and five in newborn infants at full term. Of the adults fourteen were females. The bodies were found under a variety of conditions, in rooms, hallways, cellars, et cetera. No instances of precipitate death from irritation of cervical nerves could be demonstrated.

Grasping a person by the throat is quite common in ordinary fights in attempted robbery and rape. Since it is done very quickly, often in a sudden fit of anger, the throat may be held a little too long. There is, as a rule, no premeditation. For this reason it is usually difficult for the prosecuting attorney to obtain a conviction higher than second degree murder or manslaughter, the jury often even freeing the murderer. Strangulation by a ligature, however, as, for instance, in a case of a wire wrapped around the neck indicates premeditation resulting often in first degree convictions.

In all these cases the same careful examination by the medical examiner at the scene of the crime is essential, with a description of the surroundings, the position of the body and clothing, the presence of external marks of injury, rigor mortis, and other facts.

Autopsy. External examination. Distinctive cervical scratch marks and contusions were uniformly present in the cases reported by Gonzales, with the exception of one in which hemorrhage in the deep cervical fascia and a fracture of the hyoid bone established the manner of death.

It is therefore, important, that a careful examination of the neck be made for scratch marks and contusions which from their characteristic semilunar shape may suggest finger and thumb nail injuries. Their number, situation and general arrangement may often tell whether one or both hands were used, whether a high or low manual strangulation and whether strangulation took place from in front or rear of the victim. Bruises on the back and the sides of neck may denote counter-pressure by the other hand.

Often other marks of violence may be seen around the head, face, breasts, lips, arms and hands as the victim is usually a healthy adult and unless rendered unconscious by a blow, or intoxicated or drugged, will put up a fight. Ribs may be broken due to the murderer kneeling on the body. Consciousness, however, is rapidly lost in most cases and there may be no violent struggle.

Usually external evidence of asphyxiation is well marked. The head and face are cyanotic, the face often swollen and almost black, the tongue often protrudes and is firmly caught between the teeth, the finger tips are cyanotic and often numerous asphyxial petechial hemorrhages may be found over the shoulders back and chest.

Internal examination. In my cases of manual strangulation I have used necropsy technic which was demonstrated to me several years ago by Schultze.

After a careful description of the external appearance of the body, photographs are made of the marks of violence about the neck, the body is then opened in the orthodox manner. By this examination death from asphyxia is usually established since these cases show a well pronounced general picture of asphyxia. Asphyxial hemorrhages in the pleura and pericardium, areas of acute interstitial emphysema and engorgement of the right heart with dark fluid blood is marked.

The head is next examined, often numerous asphyxial hemor-

rhages may be found in the scalp sometimes innumerable over the entire scalp. The brain is then removed. The pia-arachnoid vessels are usually distended with dark blood and occasionally petechial hemorrhages may be found in the meninges or the parenchyma of brain.

The base of the skull is then split transversely by sawing through the middle of the basilar portion of the occipital bone and the nasopharynx is opened from behind.

The position of the parts in manual strangulation in those dying with the hands of the murderer still on the throat, will be almost identical with that found in suicidal hanging. The air passages will be completely blocked by the base of the tongue being shoved backward and slightly upward, the epiglottis closing the glottis, its posterior surface in apposition to the posterior pharyngeal wall.

As to whether the tip of the tongue moves forward and protrudes between the teeth or falls backward and remains in the mouth, depends entirely on the position and direction of the compressing force in relation to the hyoid bone. During this examination accurate measurements of the parts may be taken and a photograph made. The extreme congestion of the mucosa of the nasopharynx with asphyxial hemorrhages in laryngeal mucosa and over the epiglottis, and the presence of bloody mucus or froth may be noted.

The scalp incision is then extended down the anterior borders of sternomastoid muscles and the neck dissected and explored for contusions, lacerations and hemorrhage. Fracture of the thyroid cartilage in its lateral alae, of the cricoid in its thin arch, and of the hyoid bone in its great horns may be noted. Fracture of the hyoid bone is usually seen only in high manual strangulation. In Gonzales' series, however, it only occurred four times. Smith warns against mistaking the normal joint between the body and the great horns of the hyoid, which only disappear by ossification in middle age, for a fracture. Injuries to the trachea and its rings are infrequent because of the high position of strangulation.

Usually there will be found in the deeper layers of the skin, areolar tissue, fascia and muscles over the course of the carotid vessels, hemorrhagic contusions with an occasional hemorrhage

in the thyroid or submaxillary gland. Thin sheet-like hemorrhages over the course of the carotid vessels are often seen but never have I found an internal rupture in the arteries.

The lesions in infants (infanticide) are similar to those found in adults.

In those who use the ordinary autopsy technic, the position of the parts in manual strangulation will never be found. Gonzales in a criticism of these postural findings states

The assumption that bilateral compression forces the tongue into the nasopharynx, disturbs the relations of the epiglottis and uvula, and approximates the vocal cords was not substantiated in his series. Conceding their diagnostic importance in hanging and in throttling by ligature, when the constriction remains until rigor mortis is established, their persistence after release of pressure presupposes the immediate onset of rigor and a disregard for the natural elasticity of the laryngeal cartilages.

Furthermore he claims the diagnosis of manual strangulation must rest upon the presence of such cardinal signs as the characteristic bilateral lesions of the skin, deep-seated hemorrhage and injuries to larynx or to hyoid bone. The absence of one or more of these proportionately increases the uncertainty.

I have maintained, however, that the position of the parts which have repeatedly been found in these cases has very little to do with the elasticity of the laryngeal cartilages, with perhaps the exception of the epiglottis, and that they stay in these positions due to the soft, mushy tongue being crowded back and holding them in this position.

CHOKING

Many include under the term "choking" all cases of fatal asphyxiation due to occlusion of the respiratory passages by solids, liquids or gases and also lesions in the air passages and their walls due to disease. This would include a great variety of conditions such as impaction of a foreign body in the throat, drowning, the inhalation of irrespirable gases and choking the result of cancer of larynx, et cetera.

I prefer to limit the term, in medical examiner's work, to the obstruction of the respiratory passages by solid foreign bodies.

Choking is practically always accidental. Insane persons may commit suicide by forcing solid substances into their throats. Solids have been forced into the throat of a victim for the purpose of murder. These cases, however, are rare.

Choking is common in infancy, where regurgitated milk may spill over into larynx, frighten the child and be aspirated into the smaller bronchi by spasmodic inspiratory efforts.

It is quite common in children, who have the habit of putting small objects of every description in their mouths. Attempts at speaking and swallowing at the same time, or crying out with foreign bodies in their mouths favor choking.

It is common in adults, in gourmands or hungry men, who quickly bolt large pieces of food, to have particles stick in the larynx or esophagus and press against and close the glottis. Tobacco or false teeth may lodge in the throat and produce rapid asphyxia. Smaller objects, such as fish bones, may lodge in the throat and cause asphyxia from spasm of the glottis caused by fright and edema produced by irritation.

Finally, vomitus may drain over into the larynx and, by inspiratory attempts, be aspirated into the smaller bronchi and cause asphyxiation. This is especially common in acute alcoholism and occasionally occurs during epileptic fits and in poorly administered anaesthesia. In cases of death from natural causes, a terminating asphyxia is the mode of death in many common diseases, aspirated vomitus being often found in the smaller bronchi. This must be distinguished from the stomach contents falling into the larynx due to postmortem purging; such contents will not reach the smaller bronchi.

Necropsy. Necropsy usually disclosed a more or less well defined picture of asphyxiation and the asphyxiating object is found in the respiratory tract. In persons dying with spasm of the glottis the general signs of asphyxiation may be absent.

DROWNING

Drowning is a form of asphyxial death caused by complete or partial submersion of the victim in water or other fluid. While in most cases complete submersion occurs, it is only necessary

to have enough water to cover the nostrils and mouth to produce death.

Accident, suicide or murder? From the viewpoint of the medical examiner most drownings are accidental. Homicide by drowning is rare. In New York City in a five year period (1928-1932) there were 2,257 drownings classified as accidental, only 153 suicides and only three murders.

Whether drowning is accidental or suicidal, in cases in which there are no witnesses, can only in a few instances be determined by medical examination of the body. The correct interpretation usually depends on collateral evidence, which is often vague and frequently missing. Therefore many cases which are undoubtedly suicides are signed out as accidental when there is no proof of suicide. This greatly increases the number of accidental drownings.

Necropsy. External examination. In a body recovered shortly after death any of the following findings may or may not be found: Body cold, pale with firm rigor; postmortem lividity most marked in head and chest, since the head is usually the most dependent part in water; conjunctivae suffused, eyes, prominent and often conjunctival hemorrhages; cutis anserina or goose flesh with retraction of the male genitals; bleaching, and corrugation of skin of hands and feet; the mouth usually open with presence of an extremely fine, frothy, not blood stained foam in the nostrils and mouth which increases on pressing the chest, and occasionally the presence of weeds, gravel or other foreign material firmly grasped in hands.

Internal examination. Necropsy usually shows enormously, inflated, pale, wet and soggy lungs which entirely fill pleural cavities, their anterior borders often overlapping; presence of fine, not blood stained froth and fluid in the trachea, bronchi and bronchioles with hydraulic overdistention of the alveoli with air and blebs of acute interstitial emphysema over the free borders of lungs; absence of pleural ecchymoses; lungs which on section are wet and pale and in fluid of which there may be sometimes found silt, mud, algae, et cetera characteristic of the water from which the body was recovered; the right heart and large veins

are distended with dark fluid blood, and considerable water in the stomach which often passes down into the duodenum and upper jejunum may be found.

When most of these findings are present presumptive evidence of drowning is justifiable. Smith⁸ states that only a few of the findings are, however, of distinct diagnostic value, they are:

- (1) Fine, frothy foam in mouth and nose.
- (2) Foreign material, such as weeds, stones, et cetera firmly grasped in hands.
- (3) Fine, frothy foam and fluid in trachea and bronchi.
- (4) Overinflated, soggy lungs with watery exudate on section.
- (5) Presence of water in stomach.

Most of these, however, are not absolute and they can be mimicked by other conditions. Froth and frothy fluid in mouth, nostrils and air passages may occur in pulmonary edema from a variety of causes and simulate that found in drowning; in my cases of drowning, probably due to the character of the river and ocean waters, it is rare to find weeds, et cetera in the hands of the victim. Well inflated wet lungs may be seen in pulmonary edema from many causes and may not be an outstanding feature in many cases of drowning. Finally, the victim may have drunk considerable water before submersion.

Furthermore, in bodies which have been in water for a longer period of time, these findings may entirely disappear or be masked by putrefaction so that a diagnosis as to the cause of death is impossible.

It will be seen, therefore, that since there are no absolute findings in drowning, the diagnosis depends upon the presence of one or several of the above findings which, if present, suggest death from drowning.

The blood chloride test devised by Gettler,² however, represents the only positive sign of drowning and in my experience is in most cases the only reliable information.

Normally the chloride content of the blood in the right and left chambers of the heart is about equal. When a person drowns water does not enter the lungs in any considerable quantity until the stage of terminal asphyxiation takes place, at which time

ballooning of the lungs occurs and water enters the bronchioles leading to distention of the alveoli with residual air. This period takes a considerable time to pass through and is associated with automatic respiratory efforts. Practically all drowning cases must pass through this period.

The entrance of water into the lungs dilutes the blood in the left side of the heart by a method of osmosis. No water can enter the left heart if the body is dead when thrown into the water. When drowning occurs in salt water the chloride content of the left heart will be higher than that in the right heart by as much as from 19 to 294 mg. per 100 cc. of blood (Gettler's cases) and when drowning occurs in fresh water the chloride content of the left heart will be lower than that found in the right heart.

Unfortunately in bodies which have been in water for long periods of time or in which rapid putrefaction takes place no blood can be obtained for the chemical test, the right side of the heart containing only a few gas bubbles. Such cases, especially in hot weather, often form a considerable number of the drowning cases.

Furthermore, in cases in which very little struggle occurs and consequently little water enters the lungs, the typical findings of drowning may be slight or absent, and the chemical test be inconclusive or entirely negative.

It is said that people who are intoxicated, drugged or rendered unconscious by injuries may die in water without much of a struggle. Finally the older literature contains many isolated cases in which spasmodic closure of the glottis occurs as the result of water entering the mouth and nose or from a nervous reflex due to sudden chilling of the body. Death in such cases is said to take place quickly, the victim sinking at once. These cases, however, are admitted to be rare, even by the advocates of this theory. I have always been skeptical about them and do not believe that many would stand critical review. In all such cases, however, the diagnosis of drowning would be extremely difficult and often could not be made.

Space does not permit a discussion of the physiology of drowning. Problems such as the length of time the body has been in the water, the unusual cases of submersion after death and both suicidal and homicidal submersions will be omitted.

SUFFOCATION

Asphyxia by suffocation should be limited to deaths caused by blocking of the respiratory tract from within.

Violent deaths from suffocation are often due to the presence of foreign matter in nose, mouth and respiratory tract and are usually caused by accidental falls in mud, sand, coal, dust, sawdust, flour, et cetera. They may be caused by aspiration of vomitus in acute alcoholism.

Many of the causes of suffocation have already been described under smothering as the two conditions often overlap. For instance, a person smothered by falling in mud may have both nose and mouth obstructed and at necropsy aspirated mud in the finer bronchi may be demonstrated, making the case one also of suffocation.

Suffocation occasionally occurs in children from inhalation of zinc stearate powder. I had one case of a moribund patient suffering from a malignant stricture of the esophagus, suffocated by barium given for x-ray diagnosis, the lower respiratory tract at necropsy being filled by the barium meal.

The necropsy findings in suffocation are identical with those in smothering. They are usually convincing since the general picture of asphyxia is found together with the foreign material in the respiratory tract.

Suffocation, as a terminating event in many diseases from natural causes, often occurs. Some of the common intrinsic causes being suffocation in laryngeal diphtheria (now rarely seen), edema of the larynx in chronic nephritis, the breaking of a peritonsillar abscess, hemoptysis in pulmonary tuberculosis or ruptured aneurysm.

Extrinsic causes such as suffocation in mediastinal Hodgkin's or other malignant growths of the chest, aortic aneurysms, empyemas with pleurobronchial fistula, and similar diseases, may produce suffocation.

The necropsy will clarify the situation, and it should be recalled that edema of the larynx is never so pronounced at necropsy as it has been during life.

ASPHYXIA DUE TO INTERFERENCE WITH MOVEMENTS OF
RESPIRATION*Compression of chest and abdomen*

Victims accidentally caught in a collapse of buildings, in "cave in" of excavations, or crushed between machinery, between two objects, beneath overturned automobile, or crushed in a crowd, in elevator accidents, et cetera, may die from asphyxia due to fixation of the chest and abdomen, preventing respiration.

Often no external marks of injury may be present but usually the necropsy will disclose many of the characteristic signs of an asphyxial death.

Traumatic asphyxia. Occasionally in such crushing accidents the sudden mechanical pressure may overdilate the capillaries of the face and neck causing a deep purple discoloration down to the clavicles where a sharply demarcated horizontal line occurs with normal appearing skin below it. The appearance of the patient is striking and weird. The discoloration may disappear in one to two weeks without passing through the color changes of a bruise.

Homicidal crushing. Occasionally murder is committed by kneeling on the chest of a victim, often without producing any external marks of injury. In "burking" the respiratory movements are sometimes prevented by sitting on the chest of a victim while the hands or a cloth are used over the face for the purpose of smothering.

In the middle ages, men were sometimes tortured and put to death by the placing of heavy boards and stones on the chest and abdomen, producing death by the interference of normal muscular movements of respiration.

Paralysis of respiratory muscles

Paralysis of the diaphragm and intercostal muscles occurring in such diseases as poliomyelitis, acute ascending paralysis, et cetera, may produce death by asphyxia. Fortunately the use of respirator cabinets may tide a few cases over until the disease subsides.

ASPHYXIA DUE TO PRESENCE OF IRRESPIRABLE GASES

Death by inhalation of irrespirable gases is usually an asphyxiation, and one may, with quite correctness sign, for instance, a death certificate "asphyxiation by illuminant gas, accidental, gas range, coffee pot boiled over."

Much, however, depends upon the nature of the gas inhaled as to whether death is due to a true asphyxiation, some specific poisonous action of the gas itself, or to a combination of both.

Henderson and Haggard⁶ have called attention to two types of asphyxia which may be applied to the inhalation of noxious gases.

In the first, which is caused by gases not usually considered as asphyxiants, for example hydrocyanic acid gas, respiration is quickly stopped by their poisonous effects on the respiratory centers. The tissues are deprived of oxygen and the elimination of carbon dioxide ceases. Due to the developing anoxemia and excess of CO_2 in the tissues, the effects are fatal.

In the second type, which is caused by the real asphyxiant gases, such as carbon monoxide, there is no cessation of breathing, except as a terminal event. The tissues are primarily deprived of oxygen but carbon dioxide continues to be eliminated and if excessive breathing takes place may be greatly depleted, adding to the anoxemia the condition of acapnia.

The number of gases which have caused death by inhalation either accidentally, or with suicidal or homicidal intent is large. In addition, poisonous gases play an important part in modern warfare and are beginning to be used in judicial executions.

A few of the common gases may be mentioned: Carbon monoxide, carbon dioxide (choke damp), hydrogen sulphide (stink damp), arseniuretted hydrogen (arsine), methane (marsh gas), bromine, chlorine, ammonia, the mineral acids, methyl chloride (in refrigerating systems), hydrocyanic acid gas, the anaesthetics, chloroform, ether, nitrous oxide, ethyl bromide and chloride and the war gases.

The reader should consult the classical writings of Henderson and Haggard,⁶ and the works of Webster⁹ and Smith⁸ for the

action and toxicology of these gases and the works of Herzog for the medicolegal aspects.

The enormous number of asphyxial deaths due to illuminating gas, auto exhaust and coal gas, necessitates a discussion of by far the most important of all the irrespirable gases—carbon monoxide.

CARBON MONOXIDE POISONING

Suicidal asphyxiations

The inhalation of illuminating gas is the common method of committing suicide in most civilized countries, because of its accessibility, cheapness, and supposed freedom from pain.

In New York City during a five year period (1928–1932), in a total of 7,219 suicides there were 3,003 suicides by illuminating gas or 41 percent, eight by auto exhaust and none from coal gas.

The number of suicidal asphyxiations from carbon monoxide would be much larger if one included many probable suicides, which, because of a reasonable doubt or from lack of proof, had to be classified as accidental.

Accidental asphyxiations

During this same period in New York City there were 4,677 accidental asphyxiations, 2,042 or 43 percent of these were killed by illuminating gas, seventy-nine by auto exhaust and seventy-four by coal gas.

My opinion is that most auto exhaust asphyxiations are suicides since considerable publicity in the press has warned the public concerning the danger of a running motor in small garages. In no other form of suicide can the circumstances of an accident be so mimicked by the person committing suicide as in these individual garage asphyxiations. The insurance companies usually "dig up the dirt" later when the question of payment of a double indemnity accident policy is raised.

Homicidal asphyxiations

In New York City during this same five year period there were forty-five homicides by illuminating gas, a very large number.

Most homicides in Essex County from gas have been caused by a person committing suicide who kills at the same time another person either accidentally or with homicidal intent.

The importance of carbon monoxide poisoning to a community can again be well illustrated by the New York City statistics. The same figures exist in every large city in the country.

During this same five year period in New York City there were 5,251 deaths from carbon monoxide poisoning which includes all the illuminating gas, auto exhaust and coal gas cases. This is an average of over 1,000 deaths a year. As a cause of violent death it is exceeded only by the highway accidents to which it runs a close second.

Certainly over one-half of these deaths all over the country are accidental and due to faulty and leaky gas fixtures, carelessness, intoxication, and a lack of interest by laity, hospital authorities and the medical profession in quick, competent resuscitation methods in asphyxiation.

Necropsy findings

External examination. In bodies found dead as the result of carbon monoxide whether from illuminating gas, auto exhaust or coal gas, the postmortem lividity is a characteristic bright pink color. This color is distinctive and not seen to such an extent in any other form of death. The face often appears natural, as if alive. Occasionally a similar color, although rarely so pronounced, may be seen in bodies exposed to cold and in cyanide poisoning.

If the body is found on its back, this color will be most pronounced over the back of the neck, shoulders and body. If the body is face downwards the bright red lividity will be noted over the front of the body, and will shift in a short time to dependent parts if the body is turned over, provided the blood is still confined to the capillaries and postmortem decomposition has not set in.

If the medical examiner is satisfied as to the cause of death and contemplates releasing the body for burial without a necropsy, it is often wise to obtain a specimen of blood for the toxicologist,

by aspiration from the heart. It often settles any argument as to cause of death.

When such a body is buried, it may be exhumed several months or even years afterwards, carboxy-hemoglobin may not only be detected in the blood, but even the amount of saturation estimated.

Usually a body of a person dying from carbon monoxide remains in a state of good preservation but we have, occasionally, seen very rapid postmortem decomposition.

Internal examination. In the examination of a person found dead from carbon monoxide or dying shortly after exposure, the outstanding finding is the bright cherry red color of all blood containing organs and of the blood itself. It is so pronounced that it is characteristic of carbon monoxide. The right heart is distended with this bright fluid blood. Pulmonary edema with bright pink foam in the air passages and considerable cerebral edema is often found.

If the person has been taken to a hospital and resuscitation methods, such as the administration of carbon dioxide and oxygen, restores respirations, carbon monoxide rapidly leaves the body and in a few hours an examination of the blood for carboxy-hemoglobin will be negative. The patient, however, may remain in coma and die in two or three days.

If such a body is examined after death all chemical and spectroscopic tests for carboxy-hemoglobin will be negative. The correct diagnosis in such cases often rests upon the finding of bilateral degeneration in the lenticular nuclei (Kolisko's lesions). These bilateral areas of necrosis are usually situated in the globus pallidus, are from 1 to 2 cm. in diameter, elongated in shape, brownish in color showing no hemorrhage, and grossly appear like areas of thrombotic softening. They are caused during the period of anoxemia due to the brain tissue being deprived of oxygen for too long a time. Their peculiar location is dependent upon the sharp right angles made by the nutrient arteries supplying the basal ganglia.

In two persons recently examined and who had been given intravenous injections of methylene blue in a forlorn and mis-

guided hope of saving the victims, typical areas of lenticular nucleus softening were found. On exposure to the air the nuclei turned a bright blue color, the rest of the brain remaining normal in appearance. This is a beautiful confirmation of the work of Burrows¹ on the localization of certain dyes in inflammatory areas after injection into the blood stream. The other organs which showed discoloration by the methylene blue were those chiefly involved in its elimination, the bladder and urine being a deep blue color, and the kidney pelves, ureters, and mucosa of stomach, colon and gall bladder becoming bluish on exposure to air.

Toxicological examination. Samples of blood taken at necropsy usually from the right heart, should be placed in small vials, filled to the top and tightly corked. In exhumed bodies, blood may sometimes be obtained from the cerebral sinuses when it cannot be procured easily from other locations.

These specimens should be examined by the toxicologist for the presence of carboxy-hemoglobin using chemical and by spectroscopic tests.

In medicolegal work one must no longer be satisfied with a qualitative report, since even in the blood of smokers, small amounts of carboxy-hemoglobin may be found. Quantitative estimations showing the percentage of saturation in the blood, using preferably the methods of gas analysis devised by van Slyke, is essential for a proper interpretation of the case.

In Gettler's experience, 18 to 20 percent saturation is necessary to produce symptoms, over 30 percent is dangerous and 45 to 65 percent causes paralysis and death. Contrary to other authorities he seldom finds a saturation higher than 65 percent.

ASPHYXIA DUE TO ACTION OF POISONS ON THE NERVOUS SYSTEM

Death by asphyxia is common in acute alcoholism and may occur from an overdose of morphine, hyoscine, avertin, sodium amytal, and other drugs, or from the administration of a general anaesthetic due to the direct action of these poisons or drugs on the central nervous system. It is fair to assume that numerous deaths due to drugs and anaesthetics are not reported in many communities.

In investigating anaesthetic deaths it should be constantly borne in mind that very often surgical shock and poor anaesthesia cannot be determined or properly evaluated at the postmortem table. In some instances much depends on whether the medical examiner believes there is such a condition as "status lymphaticus." Notwithstanding the opinion of Greenwood and Woods⁵ and the report of the Status Lymphaticus Investigation Committee of the Medical Research Council and Pathological Society of Great Britain and Ireland¹⁰ tending to show that it is as accurate to attribute the cause of death to "the visitation of God" as to status lymphaticus and that in the future such a condition for a cause of death will probably not be accepted by reliable authorities, I still believe persons suffering from this characteristic type of constitution die suddenly under anaesthesia and during trivial surgical or other procedures.

The correct diagnosis of most of these cases will rest upon a competent toxicologist. The methods devised by Gettler³ for recovering alcohol from the brain and the estimation of the degree of intoxication has been of enormous practical value in our work as medical examiners.

OTHER FORMS OF ASPHYXIATION

In a discussion of asphyxial deaths I have not considered an important group which would almost double the number of asphyxial deaths in any community, namely "asphyxia neonatorum."

Under this heading the following may be classed as capable of response to resuscitation properly applied: asphyxias due to cord pressure, breach extractions, malposition, deformed pelves, difficult labor, antenatal and postnatal atelectasis, prematurity, etc. This is of prime importance to investigators of infant mortality and obstetricians.

Space does not permit a discussion of the asphyxias in electric shock, lightning, conflagrations, et cetera.

The argument may be advanced that we all die of asphyxia as in many deaths from natural causes asphyxia often terminates life. The test of true asphyxial deaths, however, readily disposes

of this contention. The treatment of asphyxia is the removal of the obstruction to respiration and the application of artificial respiration.



FIG. 17. EXAMPLE OF A COMMON METHOD OF COMMITTING SUICIDE BY HANGING

The individual usually stands on a chair, box or other object, adjusts noose and kicks the support away. Occasionally, however, consciousness is lost so rapidly that he may fail to kick over support. In this case the right foot is still resting on chair, the left hanging free. (After Hofmann—*Lehrbuch der gerichtlichen Medizin*.)

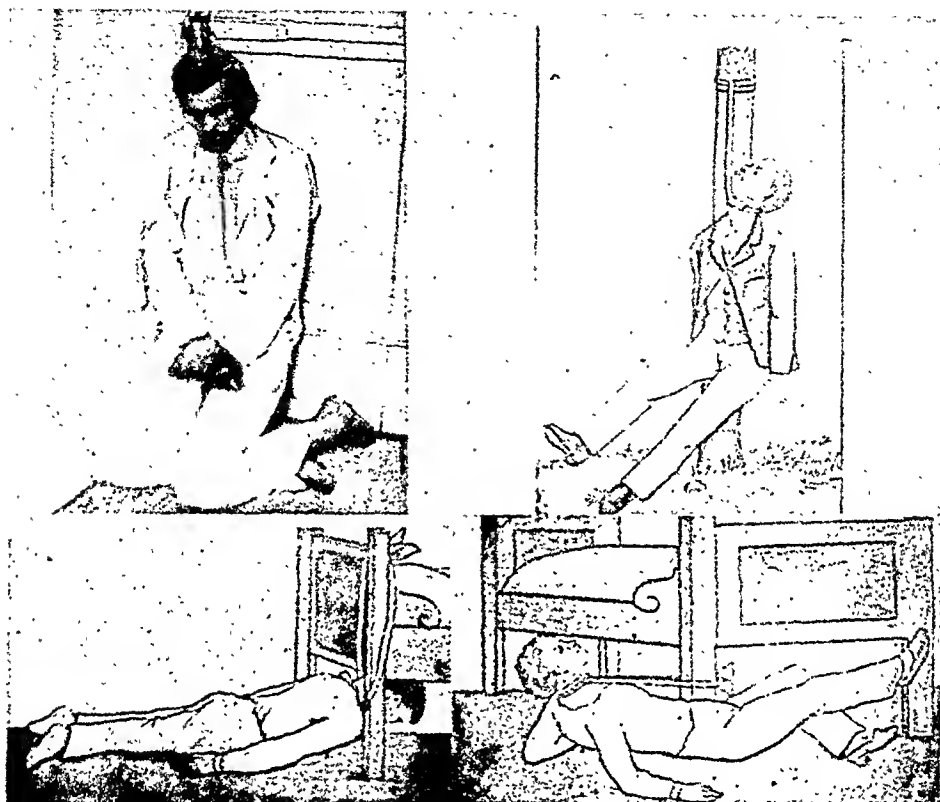


FIG. 48. EXAMPLES OF LESS FREQUENT METHODS OF COMMITTING SUICIDE BY HANGING

These cases illustrate the salient fact that complete suspension of the body is not necessary in committing suicide. Hanging in the standing, sitting or reclining position is by no means uncommon. (After Hofmann—Atlas der gerichtlichen Medizin.)



FIG. 49. TYPES OF NOOSE FREQUENTLY USED IN SUICIDAL HANGING

The picture on the left shows the use of a fixed loop. In such a case the imprint of the knot on neck or face is usually absent.

The picture on the right shows a running noose. In this case the knot which is firmly pressed into the flesh, makes a mark. (After Hofmann—Atlas der gerichtlichen Medizin.)



FIG. 50. THE FURROWS OR CONSTRICTION MARKS IN SUICIDE BY HANGING

The typical oblique furrow with its pale base and hyperemic border made by the rope, may be noted. The loop in this case was a fixed one, being so adjusted that the knot came in front of the left ear without pressing into the skin. (After Hofmann—Atlas der gerichtlichen Medizin.)



FIG. 51. MEDIAN SECTION OF NORMAL HEAD AND NECK TO ILLUSTRATE
PATENCY OF AIR PASSAGES

Note the normal position of the tongue, soft palate and uvula; the normal space between base of tongue and epiglottis; the erect position of the epiglottis; the normal space between posterior surface of the epiglottis and the posterior pharyngeal wall; the distance of at least 3 cm. between the top of the epiglottis and the interarytenoid arch, allowing full view of the vocal cords from above; the space between the soft palate, uvula and the posterior pharyngeal wall allowing free access of air from nasal passages to glottis; and the wide open structures through which air has a free passage back and forth with respiration.

All these spaces with their accurate measurement is of prime importance in the establishment of death due to strangulation, and not the ordinary data usually furnished in these cases, such as the color of face, presence of fluid blood, petechia, etc. Most of the real evidence has escaped notice because the usual autopsy technique almost entirely destroys it. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 52. MEDIAN SECTION OF HEAD AND NECK IN SUICIDE BY HANGING WITH CLOTHES LINE

Note position of tongue. Its base is pushed backward and upward by the traction of the rope, and because the point of traction is just below the hyoid bone, the tip of the tongue has been pushed anteriorly, projecting between anterior dental arcade (which in this case is formed by artificial plate-).

Note that the base of tongue is jammed back into throat completely cutting off the entrance and exit of air in respiration.

The normal space between base of tongue and epiglottis is obliterated so that the base of tongue lies in apposition with the anterior surface of the epiglottis. The epiglottis is pushed backward so that its posterior surface is in apposition with the posterior pharyngeal wall, effectively blocking the glottis.

The soft palate is pushed backward and upward, its posterior or superior surface coming in apposition with the posterior pharyngeal wall, effectively blocking the passage of air from the nasal cavities. The uvula in this case is caught and bent anteriorly against the inferior surface of the soft palate. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 53. MEDIAN SECTION OF HEAD AND NECK IN SUICIDE BY HANGING WITH LEATHER BELT

Note the position of the tongue. On account of the width of the belt there has been compression and traction on tissues above and below the hyoid bone. On account of the high compression and angle of traction, the tip of tongue remains in mouth in back of anterior dental arcade. Otherwise, note the same obliteration of the normal spaces as seen in the preceding case with effectual blocking of air from nasal cavities and from mouth. Note complete blocking of glottis by epiglottis. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 54. HOMICIDAL STRANGULATION BY LIGATURE IN COMMITTING RAPE

In this case, a stocking tightly tied running horizontally around the neck, with the knot in front, may be noted. Numerous scratches and abrasions received in the death struggle may also be noted. At autopsy it is important to remove the ligature without destroying the knot, as the manner of tying may be a valuable clue. (Office of Chief Medical Examiner—New York City.)

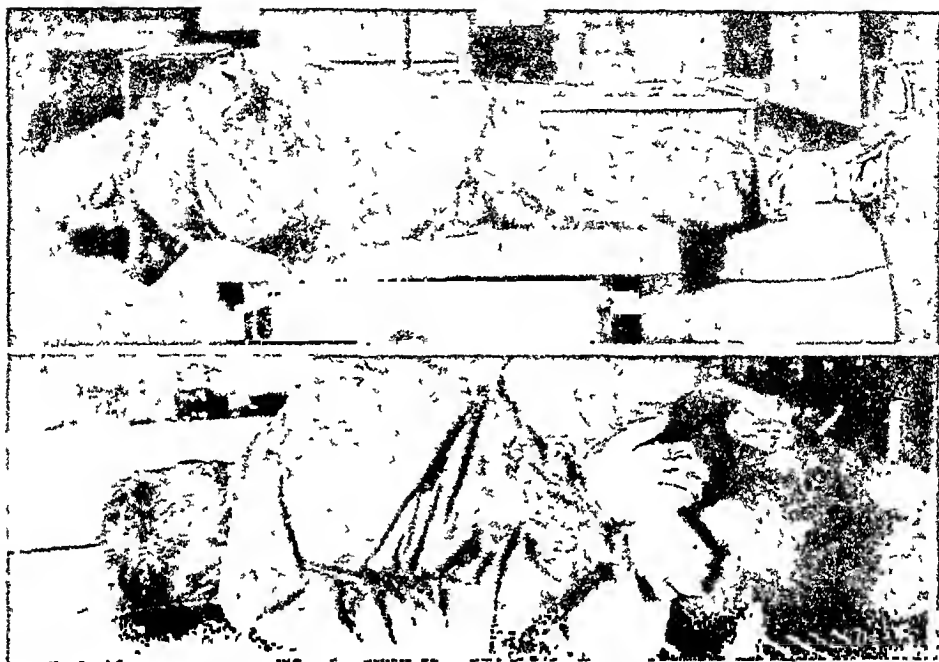


FIG. 55. SACK MURDER

The deceased was strangled, trussed up in a sack and dumped into a river. The character and method of tying the knots may be of great importance in tracking and convicting the murderer. Therefore, extreme care should be taken in removing the ropes at autopsy. (Office of Chief Medical Examiner—New York City.)



FIG. 56. MANUAL STRANGULATION—NEWBORN INFANT

Typical finger-nail impressions on the neck may be noted. (Office of Chief Medical Examiner—New York City.)

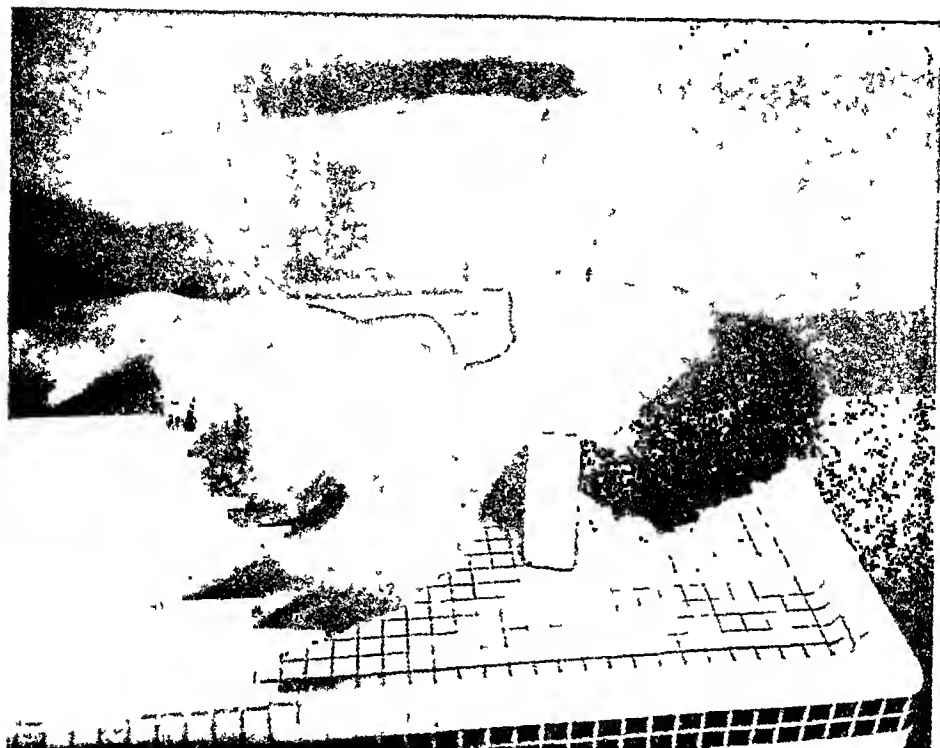


FIG. 57. HOMICIDE BY MANUAL STRANGULATION

Over left side of the neck typical finger-nail marks may be seen caused by the right hand of a giant negro. The span of the murderer's hand was so great that the marks made by the right thumb can be seen over the right clavicle. The great horn of the hyoid bone was fractured on the right side. Autopsy showed, in addition to the general signs of asphyxiation, the typical position of the soft parts of the throat seen in these cases. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 58. POSITION OF ORGANS OF MOUTH AND NECK IN MANUAL STRANGULATION
AUTOPSY FINDINGS IN CASE SHOWN IN FIGURE 57

The skull has been split horizontally by sawing through the middle of the basilar portion of occipital bone exposing the retropharynx. The obliteration of the air passages may be noted, the epiglottis being pressed back and closing the glottis, and the base of tongue forced back against epiglottis and soft palate. The vocal cords cannot be seen. Compare with normal conditions shown in Figure 59. (Office of Chief Medical Examiner, Essex County, N. J.)



FIG. 59. NORMAL POSITION OF THE ORGANS OF MOUTH AND NECK

Note the normal erect epiglottis with patent glottis exposing the vocal cords; and the normal space between the base of the tongue and epiglottis. None of these parts are forced back against the posterior pharyngeal wall. (Office of Chief Medical Examiner, Essex County, N. J.)

In preparing this sketch I have tried mainly to give my experiences as a medical examiner, disregarding the extensive literature and the reports of unusual cases.

I have often used material in an indirect manner and quoted opinions verbatim from the following books, to the authors of which I wish to acknowledge my indebtedness: *Forensic Medicine* by Sydney Smith, one of the clearest, most concise, accurate and readable books on this subject. *Legal Medicine and Toxicology* by Ralph W. Webster and *Medical Jurisprudence* by Alfred W. Herzog.

I am under deep obligation to Dr. Charles Norris, Chief Medical Examiner of New York City, for permission to use statistics or material from his office that was deemed necessary; to his deputy, Dr. Thomas A. Gonzales, for his observations on manual strangulation; and to Dr. Milton Helpert, assistant medical examiner, for the excellent photographs of many of their cases.

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APPENDIX

REPORT ON NECROPSIES*

PREPARED BY THE JOINT COMMITTEE REPRESENTING THE NEW YORK ACADEMY OF MEDICINE, THE NEW YORK PATHOLOGICAL SOCIETY AND THE METROPOLITAN FUNERAL DIRECTORS' ASSOCIATION

The joint committee began its work in 1930 and rendered a report on April 8, 1931, which was approved by the Council of the New York Academy of Medicine on May 27, 1931, and published in the *Bulletin of the New York Academy of Medicine* (7: 533, 1931). In accordance with the recommendation in section D, paragraph 3, of that report a continuing joint committee was designated to carry on the work of cooperation. Because of the unexpected large demand for copies of this original report the available reprints have become exhausted. The continuing committee therefore submits the present revision as a second report on necropsies, and recommends its adoption and printing in a periodical of wide circulation.

A. DESIRABILITY OF NECROPSY

1. All agree that postmortem examination by a pathologist is desirable; first, to provide reliable recorded information concerning the cause of death and the nature of the various disease processes; second, to confirm or amend the opinions formed by the physicians during the life of the patient, so that they may serve the next patient with greater confidence and skill; third, to reveal to the physicians continually the physical changes in the interior of the body which are associated with disordered behavior during life; fourth to provide for the advance of human knowledge con-

* Reprinted, by permission, from the *Archives of Pathology*, 14: 701-705, 1932.

cerning the nature of disease in general. It is well recognized that the practice of postmortem examination in a hospital exercises a constant influence to improve the service and to correct serious deficiencies, as well as to improve diagnosis and prevent disease.

B. COOPERATION OF HOSPITAL AUTHORITIES AND FUNERAL DIRECTORS

1. The hospital and its medical staff have not completed their service to the family on the death of a patient. They owe to the family a further service, namely, to give an account of what has occurred, together with the most accurate possible explanation. This requires that some representative member of the family come to the hospital for a personal interview and give permission for the examination of the body of the deceased. The funeral director must recognize this relationship and should not oppose the proper efforts of the hospital authorities and the physicians in the discharge of this obligation.

2. The funeral director is particularly interested in getting into his own hands: (1) the death certificate, (2) the permit to remove the body and (3) the body itself, so that he may prepare it in a satisfactory manner for the funeral ceremony. He must feel certain that nothing will arise to interfere with his plan and program. Unforeseen delay may require cancellation of contracts for transportation and various other services, thus increasing the expense and causing dissatisfaction. Unreasonable delay by the hospital, in its attempt to obtain permission for necropsy, is therefore objectionable to the funeral director. The conflict of interests in this connection requires mutual consideration and a spirit of cooperation on the part of all concerned. Disputes of this nature should therefore be adjudicated by a permanent joint committee on cooperation.

3. The funeral director or his agent must present to the hospital acceptable evidence that he has been authorized by the family to take charge of the body. The blank form employed for this purpose should conform with the requirements of the department of health.

4. Hospital employees, in general, must not give information to favored funeral directors or to any other unauthorized persons in regard to persons critically ill or dead in the hospital. It is proper for the chief administrative officer of the hospital, when requested by the family, to refer the selection of a funeral director to the office of the local Funeral Directors' Association, or, quite properly, to select one by rotation from an approved list in his own office. Such a selection must never be left to a minor employee of the hospital. Proof that a minor employee has offered recommendations of this sort should be followed by his instant dismissal from the service.

5. The hospital authorities should make certain that the necessary data for a death certificate, except those facts relating to the nature, progress and termination of the present illness, are entered on the record at the time of admission of the patient. Data such as the date of birth and the maiden name of the mother may be obtainable only with great difficulty after death of the patient. The death certificate may be filled out by a clerk using a typewriter, leaving only the diagnosis and signature to be supplied by the physician who completes the certificate.

6. Report of a death to the medical examiner should be made in those circumstances where this is legally required, and the decision to notify this official should be made at the time of death of the patient, entirely without regard to the attitude of the relatives concerning necropsy. It is improper for any member of the hospital staff to threaten to call the medical examiner if permission for necropsy is refused. Threatening or browbeating of this nature may be regarded as evidence of lack of ability to handle the situation.

7. In general, the permission for necropsy should be asked for as soon as possible after death. Often it is best to make the request at once whenever the proper relative of the deceased is present in the hospital. Reasonable consideration should be accorded to every one concerned in determining when the matter has been adequately presented and the final decision reached.

8. Arrangements should be worked out in every hospital whereby the unnecessary loss of time on the part of the funeral

director may be obviated, and the funeral director should be instructed that he will be promptly informed by telephone when the death certificate is signed and the body is ready for him.

The telephoned information in regard to the dead, particularly before a funeral director is known to have been engaged, should be given only by an executive officer of the hospital and should be carefully guarded unless the persons on the wire are personally known.

9. Interference by a funeral director with the legitimate efforts of the hospital to obtain permission for autopsy shall be regarded as a reportable grievance.

C. TECHNIC OF THE NECROPSY

1. In males, the incision is to extend from the suprasternal notch to the pubes in the midline, passing to the left of the umbilicus. In no circumstances shall the incision in males be extended further upward.

2. In females and in sailors who are to be buried in uniform, the V-shaped incision is to be used, that is, an incision extending from the acromial end of the clavicle to the xiphoid and up to the acromial end of the corresponding clavicle. The flap thus outlined must be dissected upward close to the deeper structures, and every effort must be made to prevent perforation of the skin in the process of dissection.

3. At least from one-half to 1 inch (1.2 to 2.5 cm.) of the external carotid arteries is to be left free and ligated. The internal carotids and the vertebrals are to be ligated, and at least from one-half to 1 inch of the iliaes is to be left intact and ligated.

4. The scalp is to be divided by an incision behind the ear, extending from one mastoid process to the other, as indicated in figures 2 and 3. The incision is to pass over the vertex when the hair is abundant, or somewhat posterior to this line when it is sparse. In women, the hair is to be parted along the projected line of incision to avoid cutting it. For the same reason, after the initial incision has been made, the knife should be carried in such manner that its sharp edge faces the dissector. Care should be taken not to tear or otherwise injure the scalp. The scalp is

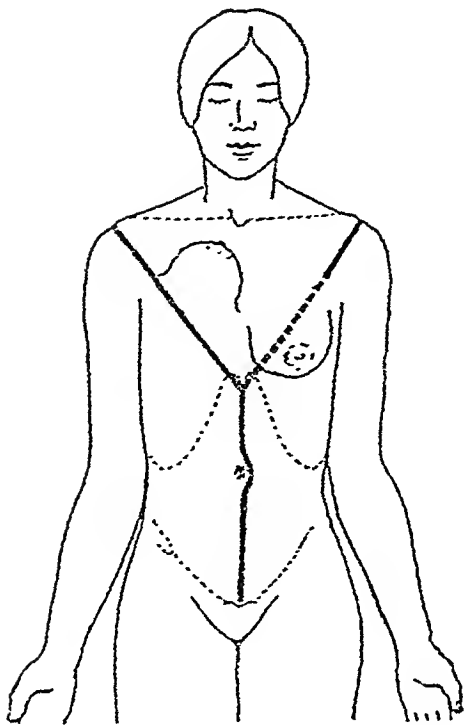


FIG. 1. DIAGRAM ILLUSTRATING INCISIONS IN TRUNK

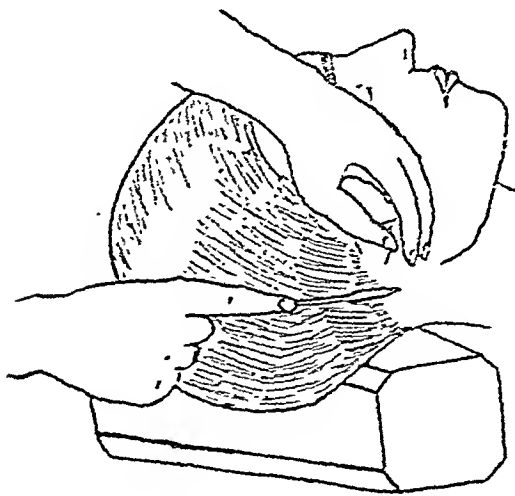


FIG. 2. INCISION IN SCALP

reflected backward and forward, so that the calvarium is exposed anteriorly slightly above the frontal eminences and posteriorly somewhat behind the occipital protuberance.

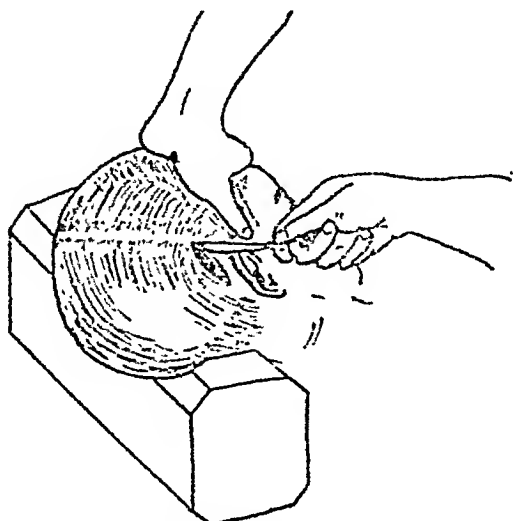


FIG. 3. INCISION IN SCALP

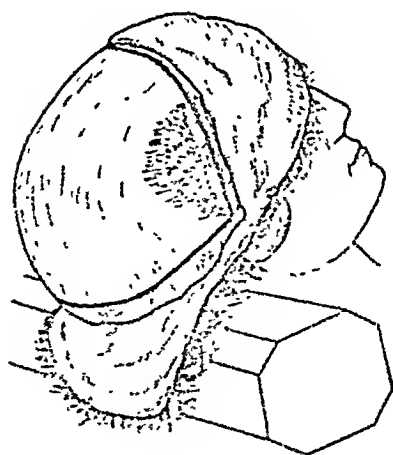


FIG. 4. REMOVAL OF SKULL CAP

Before the skull is sawed, the line through which the saw is to be carried is to be mapped out with the aid of a sharp instrument (fig. 4). The temporal muscles are to be cut on a plane parallel

with the projected line (fig. 4) to preserve stumps on either side long enough to provide for suturing and immobilization of the replaced calvarium.

5. The removal of the skull cap is to be planned and carried out in such a manner as to insure its secure approximation. This is best accomplished by sawing in two intersecting lines which meet at an obtuse angle behind the ear (fig. 3), the anterior incision commencing at the level of the hair line.

6. Before closing the cranial cavity, every effort should be made to provide against leakage. This is best carried out by the following procedures: (a) by ligating the carotid and vertebral arteries, (b) by plugging the foramen magnum tightly with cotton and (c) by filling the cranial cavity with oakum.

7. In suturing the skin a moderately small needle should be used so as to avoid leakage and disfigurement.

8. After the autopsy is completed, the body is to be delivered to the embalmer in a thoroughly clean condition—the skin washed, all cavities thoroughly sponged and dried and no source of leakage allowed to remain.

9. After the completion of the autopsy, the embalmer is to be allowed the use of the autopsy room for the preparation of the body for burial, provided that this does not conflict with the immediate use of the room for another autopsy and provided also that the embalmers leave no cleaning to be done by the hospital employees.

GEORGE BAEHR, M.D., *Chairman.*



RECOGNITION OF THE ALLERGIC STATE BY TISSUE EXAMINATION

THE RESPIRATORY TRACT AND THE NASAL SINUSES*

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The scope of the subject presented in this work is limited to the types of allergic disease which Coca labelled "atopic" and which corresponds to certain forms of human hypersensitiveness with an hereditary basis such as asthma and hay fever. There are several shock organs (as Doerr designated those organs which show the allergic lesions) in human hypersensitiveness consisting of the skin, the conjunctiva, the nose, the lung, the gastro-intestinal tract, and possibly the meninges, the urinary bladder and the retina. Of these organs the basic tissues of the more commonly encountered diseases may be placed into three groups: (1) the mucosa of the respiratory tract and the accessory nasal sinuses, (2) the skin and (3) the mucosa of the gastro-intestinal tract. Probably and largely because of the difference in the histological structure of these tissues, there is a modification in their allergic pathological picture. The work here presented will consider the histopathology of the respiratory mucosa and that of the accessory nasal sinuses as exemplified by the clinical conditions of allergic asthma, hay fever and sinusitis.

The literature contains reports of forty-seven necropsies performed on people dying of a presumptive allergic asthma. The earlier reports were summarized by Huber and Koessler³ and later contributions were made by Kountz and Alexander,⁴ Steinberg and Figley,⁷ and Macdonald⁵ with references to other reported cases. Critical analyses of these forty-seven necropsy reports

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

(Huber and Koessler, Steinberg and Figley, and Rackemann⁶) indicate that only some sixteen of them were actually instances of allergic asthma and a still smaller number were free of complicating conditions which might have altered and confused an unfamiliar pathological picture. Those who had the opportunity to study at first hand the morbid anatomy of cases of true allergic asthma were uniformly impressed with the peculiar pathologic changes in the lungs. Either by the implied written word or by personal communication to the author, some of these observers expressed their belief that there is a distinct histopathological picture associated with allergic asthma. If, however, a survey is made of all the reported cases without regard to their etiological basis, a contrary view may be formulated. Walzer⁷ analyzed the pathological findings of thirty-three cases and concluded that there is no uniformity in the findings nor is there a clue to the pathology of bronchial asthma and that the changes found are due to secondary bronchiectasis and emphysema. This view is not in accord with that expressed by most of the other observers.

Hansel⁸ reported that the pathological changes in the nasal mucosa were similar to those in the bronchi of asthmatic individuals. Kountz and Alexander removed a section of the nasal mucous membrane from one of their cases of allergic asthma and found that the histopathological picture there and in the bronchi were alike. These observations explain the frequent clinical manifestations of sneezing and coryza preceding an asthmatic attack. Matson in discussing the presentation of Steinberg and Figley stated that the tissue of the accessory nasal sinuses in asthmatics contained eosinophiles and edema with an hyperplasia of the lining epithelium including goblet cells. Tissue removed from all the accessory sinuses of the nose in one of my cases of allergic asthma presented pathological changes in the nose similar to those observed by Hansel and Kountz and Alexander. Hansel studied the histopathology of the nasal and sinus mucosa in hay fever, vasomotor rhinitis and bronchial asthma. The changes he observed in the three conditions were identical. It is therefore apparent that in the individuals with allergic asthma,

the entire respiratory tract including the sinuses participate in the altered histological structure and that these changes throughout the system are similar in character. It may also be inferred that the allergic (atopic) state, in whatever manifestation it occurs in affecting the respiratory and sinus mucosa (asthma, hay fever, sinusitis, rhinitis) is characterized by a single and a constant histopathological picture. The purpose of this work is to present this histopathology and further evidence to substantiate these contentions. The work is based on a study of three necropsies on people dying of allergic asthma and an analysis of true allergic asthma cases in the literature. The study also includes the examination of accessory sinus tissue from thirty cases of sinusitis including nasal tissue in those with hay fever.

PATHOLOGY OF LUNG IN ALLERGIC ASTHMA

The three cases of allergic asthma which form the basis of the pathological description to follow were definitely established instances of allergy as determined by immunological and hereditary criteria. Two other cases were left out of consideration because they lacked either the immunological or the hereditary evidences and also because of complicating infectious manifestations. The lungs only will be described since the other organs did not reveal morbid changes which could be attributed to allergic disease.

Gross appearance

The lungs were voluminous but were decreased in weight to a little more than half of the normal. The surface lobules were enlarged. In the case of long standing disease, there were patches of bullous emphysema. The cut surface was dry, very spongy with large visible alveoli. An occasional area was of a dull red color, the alveoli were obliterated and the tissue was rubbery to touch. These were areas of atelectasis. The walls of all the bronchi were perceptibly thickened and were gray white in color. The lumina of the bronchi were filled with gray, frequently concentrically arranged plugs. The consistency of these plugs varied. They were soft, mucoid and fairly easily removable

but more frequently they were stony hard and could not be dislodged. Bronchi of every calibre were involved.

Histological appearance

The lining cells of the bronchi which were normally ciliated, columnar in type with interspersed goblet cells, had undergone several changes. In the bronchi with hard plugs, the columnar cells were reduced to very short cuboidal with dense nuclei which occupied the entire cell. The goblet cells were few in number or entirely absent. The bronchi with soft plugs or with a lumen incompletely filled had several layers of columnar epithelium with numerous goblet cells. The nuclei of the columnar cells were located at the margins of attachment, they were vesicular and showed prominent nucleoli. The goblet cells were distended with circular or ovoid areas either clear or containing mucinous material.

The basement membrane was considerably thickened and structureless in character. It gave a hyaline reaction to Unna's stain. The average width of the basement membrane was 0.025 mm. irrespective of the calibre of the bronchus. The basement membrane of the smaller bronchi was of the same width or wider than that of the larger bronchi. The mucosa contained a variable degree of edema and cellular infiltration. Eosinophiles composed from 15 to 85 per cent of all cells, the remaining were lymphocytes. There was a varying proportion of the mononuclear and the polymorphonuclear eosinophiles with a tendency to a greater number of the mononuclear type. The muscle layer was also infiltrated by similar cells. There was a separation of the muscle bundles by the edematous fluid. The muscle tissue was distinctly increased in amount. Measurements demonstrated their increase to be from five to ten times that of normal. There were no apparent changes in the muscle fibers except for a possible increase in width and more than the usual vesicular nuclei. The musculature was smaller in amount in cases in which the disease was of a brief duration. The elastic fibers appeared broken.

The mucous glands for the greater part were markedly increased in size and were filled with a large amount of mucus. The

diameter of an average gland was 0.18 mm. as contrasted with 0.058 mm. for a normal. The cells composing the mucous glands were indistinguishable with only an occasional outline to indicate the presence of a cell wall. The nuclei were dense, flattened and were crowded to the base of the cell. An occasional basement membrane was thickened and hyalinized. There was an apparent hypersecretory activity of the glands but the changes in the cytoplasmic structure were probably reversible since the nuclei though compressed did not show disintegrative changes. The tissue surrounding the glands was edematous and contained a cellular exudate similar to that in the mucosa. The cartilage

CHART 1

A SUMMARY OF THE ESSENTIAL CHANGES CHARACTERIZING THE HISTOPATHOLOGY OF THE LUNGS IN ALLERGIC ASTHMA

1. Emphysema.
2. Edema of the bronchial wall.
3. Hypertrophy of the bronchial muscle.
4. Eosinophilic infiltration of the bronchial wall.
5. Hypertrophy and hypersecretory activity of the mucous glands.
6. Increased amount of mucus in bronchial and gland lumina.
7. Hypertrophy and hyalinization of the basement membrane.
8. Hyperplasia and hypersecretory activity of the goblet cells of the bronchi.
9. Degenerative changes of the cartilage cells of the bronchi.

plates showed degenerative changes with decalcification in some places and an apparent increase in the calcium in others. The lumina of the bronchi contained strands and structureless masses of mucus with enmeshed desquamated epithelial cells, lymphocytes and eosinophiles. In some of the bronchi, especially those with cuboidal cells, the lumen contents were adherent to the lining cells. There were frequent herniations into the wall of the bronchi. The lumina were actually increased in diameter as demonstrated by comparative measurements with normal bronchi. This increase was apparently due to an excessive amount of mucus secreted into the lumen. Although there was an actual increase in the diameter of the bronchial lumen, there was no functional advantage because of the plugging by mucus.



FIG. 1. A. Section of lung in allergic asthma. A bronchus filled with a moderate amount of mucus. Hyperplasia of the lining cells. Thickened and hyalinized basement membrane. Cellular infiltration, the cells are predominantly eosinophilic. Hypertrophy of the muscular structure.

B. A higher power photograph of a part of a bronchus from a case of allergic asthma. a. Mucous secretion in the lumen. b. Cuboidal lining cells. c. Thickened and hyalinized basement membrane.

C. A high power photograph of a part of a bronchus from a case of allergic asthma. a. An eosinophile representing 80% of the total number of infiltrating cells.

The alveoli were distended and many of the septa were broken allowing many alveoli to intercommunicate. The septa were considerably narrower than normal. The capillaries and the blood vessels were dilated and contained large amounts of blood. This emphysema was apparently of long standing. The essential changes are summarized in chart 1.

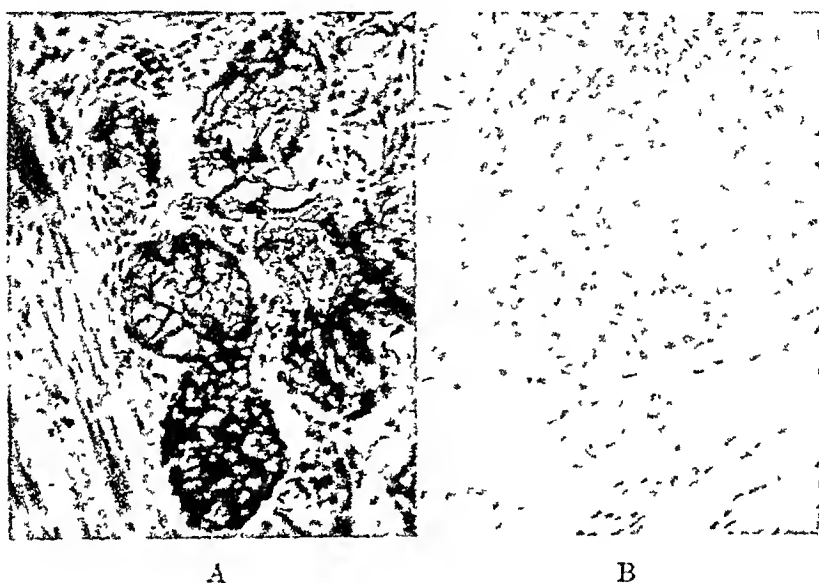


FIG. 2. A. Mucous glands from the wall of a bronchus of a lung in a case of allergic asthma. The glands are increased in size. The lining cells are not apparent. In place of the cells there is a considerable amount of mucus which also fills the lumen. There is a cellular exudate surrounding the glands. The cells are predominantly eosinophilic.

B. Mucous glands from the wall of a bronchus in a case of chronic bronchitis. The glands are normal in size. The lining cells are distinct. The nuclei are preserved. There is no mucus in the lumen.

PATHOLOGY OF THE MUCOUS MEMBRANES OF THE NOSE AND THE ACCESSORY SINUSES IN HAY FEVER AND ALLERGIC SINUSITIS

The clinical histories and the pathological findings were correlated in thirty cases in which the sinus and in many the nasal tissue were removed. In more than half of the cases, the sinus operation was performed in several stages allowing a study of the tissue during various stages of activity of the disease. Because

the histological structure and the pathological changes are alike, the sinus and nasal tissue will be considered together.

Gross appearance

The tissue was boggy and occasionally polypoid. The color was a muddy gray pink. There was frequently a thick, gray,

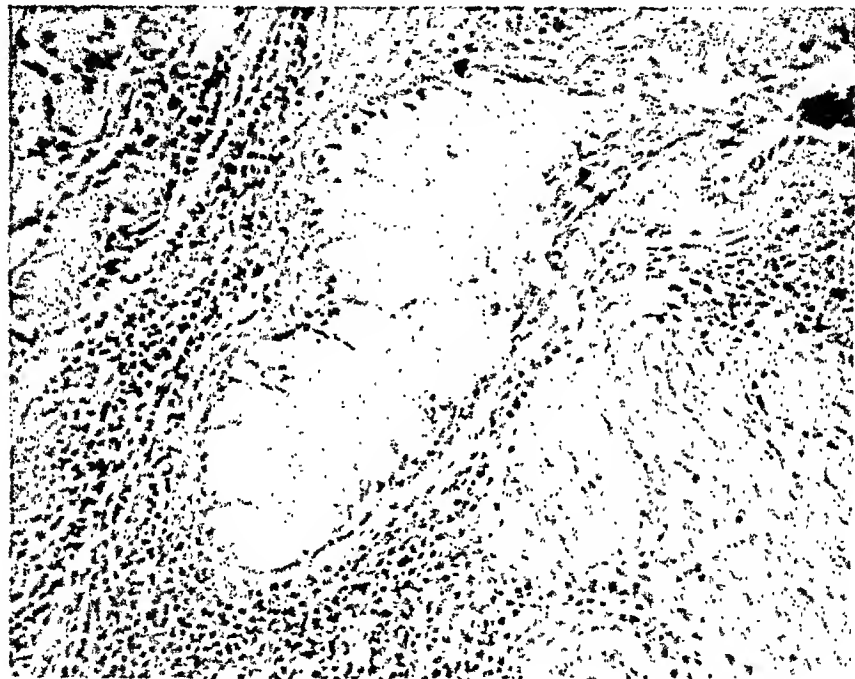


FIG. 3. A BRONCHUS FROM A LUNG FROM A CASE OF ALLERGIC ASTHMA.
HERNIATION OF A BRONCHUS INTO THE WALL.

The lumen is filled with mucus. The lining cells are cuboidal in type. There are outlines of numerous goblet cells. The basement membrane is thickened and hyalinized. The cellular infiltration consists of eosinophiles and lymphocytes.

mucoid substance on the surface and occasionally streaky gray mucus in strands or small masses. The cut surface was gray, glaucous, glistening and very moist. There were small pin points of darker gray areas which correspond to a group of hypertrophied tubules.

Histological appearance

The lining cells which are normally ciliated columnar were frequently reduced to low cuboidal. The nuclei were dense and were located at the attached margin of the cell. Associated with this change of the epithelium, there was a complete disappearance of the goblet cells. In other instances and frequently in the same field there was a marked increase in size and in number of the goblet cells with preservation and hyperplasia of the ciliated columnar epithelium which was frequently present in two or three layers. The goblet cells were distended with mucus. The nuclei were elongated and compressed to one side of the cell wall. The basement membrane was thickened and hyalinized. The average variation in the width was 0.1 to 0.2 mm. Parts of the membrane in the same tissue remained normal and the width varied in the same microscopic field. In the clinically early cases of recent origin, there was thickening but preservation to some extent of the fibrillar structure. In the older cases, the fibrils had disappeared and the membrane presented a homogeneous appearance.

The mucous membrane was edematous. The edema varied proportionally with the acuteness of the condition. The fluid had a bluish tinge in a hematoxylin-eosin preparation. The fibroelastic tissue was compressed and in places was granular. There was a variable cellular infiltration. The greater number of cells were eosinophiles with a variable proportion of the polynuclear and the mononuclear types. The eosinophiles constituted from 15 to 90 per cent of all cells, the number depending upon the acuteness and the severity of the clinical state. Lymphocytes, endothelioid cells and an occasional plasma cell composed the remaining cellular constituents. Fibroblastic proliferation was present to a very slight extent and in a very few instances.

The mucous glands were increased in size and were present in apparent larger numbers. The average individual hyperplastic gland measured 0.15 mm. as contrasted to the dimensions of a normal of 0.06 mm. The glandular lumen was dilated and filled with mucus. The lining cells were for the greater part not ap-

parent and only a thin line showed the presence of the cell wall. Some of the glands contained a fairly well defined cell wall and the cytoplasm had globules of mucus. The nuclei were located

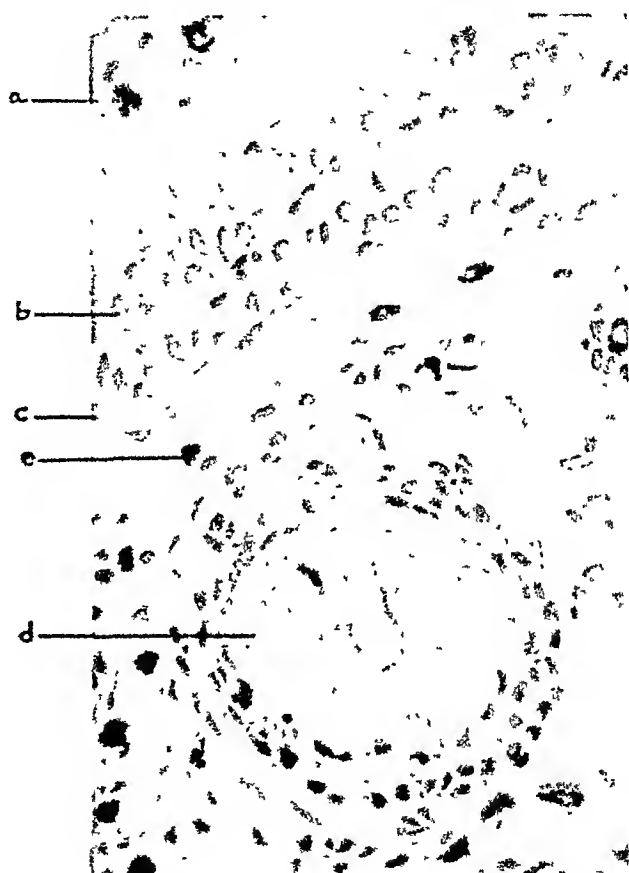


FIG. 1. FRONTAL SINUS TISSUE FROM A CASE OF ALLERGIC SINUSITIS WITH A HISTORY OF ALLERGIC ASTHMA

a - Lumen of sinus containing mucus and eosinophiles. b - Several layers of lining cells and goblet cells. c - Thickened and hyalinized basement membrane. d - A mucous gland which is increased in size, but the lumen contains a large amount of mucus and flattened lining cells. e - Mucosa with edema and eosinophilic infiltration

at the base of the cell, they were compressed and were stained deeply. The cartilage cells took a deep hematoxylin stain in

some places and in others there was an evident diminution of calcium. The cytoplasm and the nucleus showed degenerative changes.

An analysis of the clinical state of the patient and the correlation to the histopathology showed evidence that the histological changes vary with the clinical state of the patient. These variations are shown in chart 3.

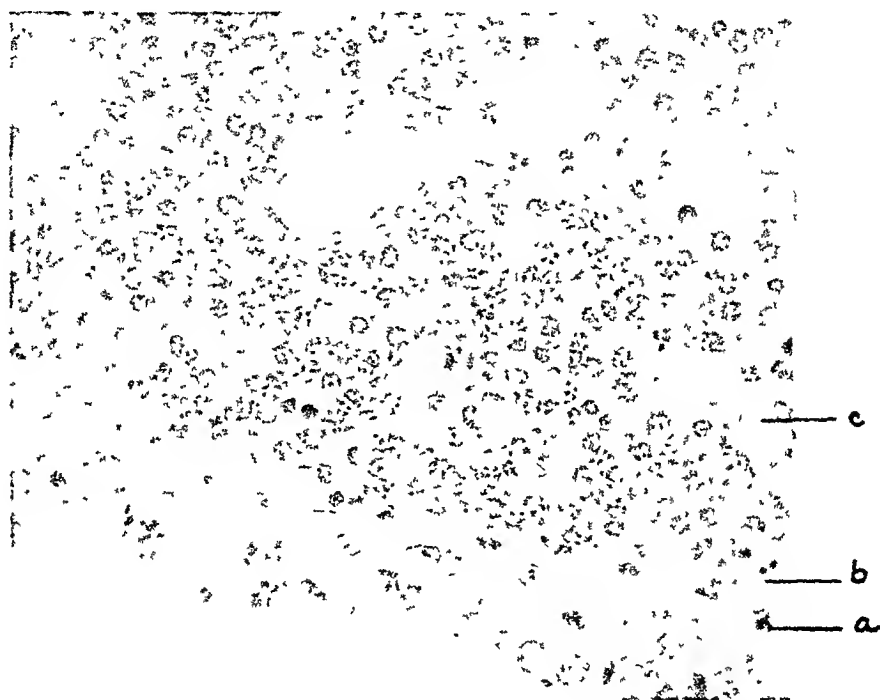


FIG. 5. TISSUE FROM THE FRONTAL SINUS FROM A CASE OF CHRONIC INFECTIOUS SINUSITIS

a—Several layers of lining epithelium which tend to assume a squamous type. b—An indistinct basement membrane. c—A mucosa diffusely infiltrated with plasma cells and a sprinkling of polymorphonuclears. Contrast this histopathological picture with that in Figure 4 representing an allergic state.

SUMMARY

There is a distinct histopathological picture of the mucosa of the entire respiratory tract and of the accessory nasal sinuses associated with the allergic (atopic) state. The morbid changes are of a similar nature in the atopic conditions of asthma, hay

CHART 2

A SUMMARY OF THE ESSENTIAL CHANGES CHARACTERIZING THE HISTOPATHOLOGY
IN ALLERGIC SINUSITIS AND HAY FEVER

1. Edema.
2. Hyperplasia and hypersecretory activity of the goblet cells.
3. Thickening and hyalinization of the basement membrane.
4. Eosinophilic infiltration.
5. Hypertrophy and hypersecretory activity of the mucous glands.
6. Presence of mucus in lumen of sinus and glands.

CHART 3

HISTOPATHOLOGY OF THE VARIOUS STAGES OF ALLERGIC SINUSITIS

STAGE	MUCOUS GLANDS	EDEMA	GOBLET CELLS	BASEMENT MEMBRANE	AMOUNT OF MUCUS	EOSINO-PHILIA
Acute Stage	Hyperplasia Hypertrophy Hypersecretory activity	Moderate to marked	Hyperplasia Hypersecretory activity	Slightly thickened, granular or homogeneous	Moderate to marked	75-90 per cent
Chronic Stage	Hyperplasia Hypertrophy Hypersecretory activity and dilatation of glandular lumina	Moderate to marked	Hyperplasia Hypersecretory activity	Greatly thickened and homogeneous	Moderate to marked	35-90 per cent
Remission	Hyperplasia Little or no hypertrophy No secretory activity	Very slight	Not apparent	Moderately to greatly thickened and homogeneous	Little or none	15 per cent

CHART 4

SUMMARY OF THE ESSENTIAL HISTOPATHOLOGICAL CHARACTERISTICS OF
ALLERGIC (ATOPIC) MUCOUS MEMBRANES OF THE ENTIRE
RESPIRATORY TRACT AND ACCESSORY NASAL SINUSES

1. Hypertrophy and marked secretory activity of the mucous glands.
2. Presence of large amount of mucus in lumina.
3. Eosinophilia—from 15 to 90 per cent of all cells.
4. Edema of tissue.
5. Thickening and hyalinization of the basement membrane.
6. Hyperplasia of goblet cells with hypersecretory activity.

fever and rhinitis (chart 4). In asthma, in addition to the lungs, the rest of the respiratory tract including the nose and almost invariably the accessory sinuses show these morbid changes. This constant pathological picture of the respiratory, nasal and sinus mucosa permits recognition of the allergic (atopic) state involving these organs.

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THE SPECIFICITY OF THE TEST FOR ALCOHOL IN BODY FLUIDS*

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The subject of alcohol and its effects on the human body has recently assumed unusual interest, and the public has been so overwhelmed with political and business propaganda that the results of scientific investigation have been thrust into the background. The question has assumed such emotional characteristics that it provokes reactions similar to those accompanying discussions of politics and religion.

Even the diagnosis of drunkenness is no longer limited to the medical profession, a supreme court having ruled that any person of ordinary intelligence is competent to testify as to the intoxication or sobriety of a person. This is an indictment of the medical profession, in recognition of the fact that its members have failed to diagnose drunkenness to the satisfaction of courts and juries. Even though the accused may have been visibly intoxicated, a clever lawyer can point out that there is no symptom of alcoholic intoxication which may not be simulated by some other pathological condition, and acquittal usually follows.

Many observers, among whom are Nicloux¹ and Nowicka,⁵ Widmark,⁷ Southgate³ and Carter,⁶ Bogen,¹ McNally and Embree⁴ have shown that a chemical test for alcohol furnishes the only constant finding in all cases of alcoholic intoxication, and also allows the examiner to state with assurance that at least a certain amount of alcohol was taken into the body. We³ have also confirmed the fact that the intensity of intoxication fairly closely parallels the per cent of alcohol in the blood or urine, regardless of tolerance, age, weight, or food consumption. This was shown by

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controlled experiments on human beings, and practical confirmation was furnished by my co-worker Dr. Benjamin Halporn, who was able to estimate the amount of alcohol in the urine of persons accused of driving while drunk with remarkable accuracy from the symptoms alone, his estimate being later checked by chemical analysis.

Although the test has been used by us chiefly as a means of confirming evident drunkenness, it also proves of great value in collecting information about individuals who show no symptoms of intoxication, having been suddenly sobered by an accident or contact with the police. Also cases in which the injuries of persons mask the effects of alcohol, and cases of fatal accidents will yield valuable information on chemical analysis for alcohol.

We have had several cases in which the chemical test aided in the differential diagnosis of coma, one of which will be summarized.

A middle aged man was sent to the hospital in a state of coma. According to the history given by relatives he had been drinking and had fallen striking his head. The x-ray revealed a fracture through the parietal bone and right mastoid. The condition of the patient was alarming and the surgeon wished to know whether or not to operate. A catheterized specimen of urine obtained two hours after the accident revealed 0.42 per cent alcohol, and spinal fluid examined one hour later revealed a pressure of 300 mm. water, normal rise on jugular compression; three lymphocytes and 20 erythrocytes; globulin ++; and alcohol 0.38 per cent. The conclusion that alcohol could have been responsible for most of the alarming symptoms was later on justified, the patient making a rapid recovery.

So meager is the information regarding alcohol flavored accidents, that the state of Pennsylvania reports drunkenness as associated with fatal auto accidents as but 1 per cent for 1931. These figures have suggested that the rôle of alcohol has not been properly evaluated, and we are now engaged in a survey to determine the relationship of alcohol to auto accidents, having succeeded in collecting data on fifty accidents (table 1). While the number of cases is, of course, too small to permit drawing of conclusions, it at least suggests the importance of a nation-wide survey.

Obviously such work would be useless unless we had a simple.

TABLE 1

PRELIMINARY INVESTIGATION OF FIFTY CONSECUTIVE AUTOMOBILE ACCIDENTS

	NUMBER	INJURED	KILLED
"Alcohol accidents".....	32	71	4
No alcohol involved.....	18	23	0

TABLE 2

STRONG PERMANENT STANDARDS

PER CENT ALCOHOL BY WEIGHT	ALCOHOL BY WEIGHT	WATER
		cc.
0.0	<i>55</i>	1.00
0.05	0.50 cc. of 0.10 per cent	0.50 <i>75</i>
0.10	<i>28</i> 1.00 cc. of 0.10 per cent	0.0 <i>100</i>
0.12	0.60 cc. of 0.20 per cent	0.40
0.14	0.70 cc. of 0.20 per cent	0.30
0.16	0.80 cc. of 0.20 per cent	0.20
0.18	0.90 cc. of 0.20 per cent	0.10
0.20	1.00 cc. of 0.20 per cent	
0.22	0.73 cc. of 0.30 per cent	0.27

To each tube add 3 cc. N/15 $K_2Cr_2O_7$ (0.33 per cent in 50 per cent H_2SO_4). Place tubes in boiling water four minutes and seal.

TABLE 3

WEAK PERMANENT STANDARDS

PER CENT ALCOHOL BY WEIGHT	ALCOHOL BY WEIGHT	WATER
		cc.
0.0	<i>1.0</i>	2.0
0.005	1.0 cc. of 0.01 per cent	1.0
0.010	<i>0.4</i> 2.0 cc. of 0.01 per cent	0.0
0.013	0.52 cc. of 0.05 per cent	1.48
0.016	0.64 cc. of 0.05 per cent	1.36
0.019	0.76 cc. of 0.05 per cent	1.24
0.022	0.88 cc. of 0.05 per cent	1.12
0.025	1.00 cc. of 0.05 per cent	1.00
0.028	1.12 cc. of 0.05 per cent	0.88
0.031	1.24 cc. of 0.05 per cent	0.76
0.034	1.36 cc. of 0.05 per cent	0.64
0.037	1.48 cc. of 0.05 per cent	0.52
0.040	1.60 cc. of 0.05 per cent	0.40

Add 1 cc. $K_2Cr_2O_7$ reagent to each tube. Place tubes in boiling water bath twelve minutes.

specific test, and could be assured that the alcohol in specimens would not change appreciably for several days. Many methods for determining alcohol have been used, but one of the simplest is the reduction of potassium dichromate with a change of color from orange to blue, the reading being obtained by comparing the color with standards prepared by adding known amounts of alcohol to the reagent. The technic previously published has been improved.

TECHNIC OF TEST

Distill mixture of 10 cc. of urine with about 10 cc. of half saturated picric acid containing about 10 per cent tartaric acid, collecting the first 10 cc. of the distillate and mix. In separate tubes similar to those used for the standards, place 1 cc. in one, and smaller measured amounts in the others, making the volume up to 1 cc. in each case. Add 3 cc. of the $K_2Cr_2O_7$ reagent to each tube, and place in boiling water bath four minutes. Compare colors with those of the standard scale. Divide the reading by the fraction of a cubic centimeter of distillate used, which gives the percentage of alcohol by weight. The use of several tubes permits close checking of the results and gives greater opportunity for having readings on the scale.

If results are too low to be read, use the weak standards, using 2 cc. of the distillate and known smaller amounts, bringing the volume to 2 cc. in each case, add 1 cc. of the reagent and place tubes in boiling water twelve minutes.

If blood is being tested take 2 cc. of whole blood, plasma, or serum (all give the same results), add about 15 cc. of the picric-tartaric reagent, and collect the first 10 cc. of the distillate. This is tested on the weak standard scale and the result multiplied by 5.

ACCURACY OF TEST

The color changes produced are so definite that two technicians can consistently check results within 0.01 per cent, when the strong standards are used, and 0.002 per cent when the weak standards are used.

Using a 500 cc. Pyrex Florence flask, heating with a flame through an asbestos pad, and using a vertical condenser, not of the spiral type, no measurable loss of alcohol could be found after distillation.

SPECIFICITY OF TEST

Mention is frequently made of traces of a reducing substance found normally in body fluids, which might be confused with

alcohol. This substance has recently been proved to be ethyl alcohol by Gettler² and our experiments on non-drinkers have consistently shown the amount to be below 0.005 per cent.

Authors have repeatedly pointed out that the reduction of potassium dichromate is not necessarily specific for alcohol, and have named ether, salicylic acid, chloral, chloroform, acetone and lactic acid as possible sources of error.

Our previous paper has given results of experiments which indicated that chloroform, ether, chloral hydrate, salicylates, and acetone could not be mistaken for ethyl alcohol, since they could not be present in sufficient amounts to appreciably reduce $K_2Cr_2O_7$ and, furthermore, gave high readings with the refractometer thus giving evidence of their presence.

The fact that ether does not interfere with the test is further demonstrated by testing the blood from a patient who had been having an ether anaesthetic for one hour. Here the refractometer reading corresponded to 0.03 per cent of alcohol, but the chemical test read 0.002 per cent.

Lactic acid readily reduces $K_2Cr_2O_7$ and does not have as high a refractive index compared to its reducing properties as the other substances studied. For example 1 per cent lactic acid by weight corresponds to 0.60 per cent alcohol by the reduction test and to 1.97 per cent alcohol when tested with the refractometer. However this substance does not appear in the distillate, and need not be considered as a source of error.

Salicylates failed to appear in the distillate and can also be disregarded.

Our experiments have clearly shown that none of the substances said to interfere have any practical bearing on the specificity of the test. Also the use of the refractometer may be omitted, although it is a convenient method of checking the results, and is a particularly efficient weapon to confound the attorney for the defense who has been delving into the literature where he has found no mention of this instrument.

PRESERVATION OF SPECIMENS

We have already shown that specimens of urine, even from diabetics, show no appreciable change in alcohol content for at

least twenty-four hours when left at 37.5°C. Also specimens preserved with benzoic acid retain their alcohol for months, and no fermentation is produced even in the presence of dextrose and yeast.

Continuing these experiments with blood, it was found that blood containing potassium oxalate, sodium citrate, and benzoic acid remained unchanged as far as alcoholic per cent was concerned for at least five days. Sodium fluoride proved to be the best preservative, the alcohol decreasing only from 0.30 to 0.28 per cent in a month, and showing no change for ten days. All specimens were kept at room temperature 20° to 30°C. (see table 4).

TABLE 4

KEEPING PROPERTIES OF BLOODS TO WHICH HAVE BEEN ADDED PRESERVATIVES
AND ANTI-COAGULANTS

(Readings represent alcohol in per cent)

DATE	SODIUM FLUORIDE	POTASSIUM OXALATE	SODIUM CITRATE	BENZOIC ACID
April 25.....	0.30	0.30	0.40	0.37
April 27.....	0.30	0.30	0.40	0.37
April 29.....	0.30	0.30		
May 1.....	0.30	0.30	0.40	0.35
May 5.....	0.30	0.10	0.40	0.35
May 11.....	0.28		0.28	0.30
May 22.....	0.27		0.008	0.005
May 27.....	0.28			

SUMMARY AND CONCLUSIONS

A simple and specific test for alcohol in body fluids is described and its specificity has been demonstrated.

Specimens of blood and urine may be preserved for at least a month. For blood use sodium fluoride, and urine, benzoic acid.

The importance of the test lies in its ability to confirm a diagnosis of drunkenness for medicolegal purposes, as well as to give valuable information in differential diagnoses.

A preliminary survey of persons injured or killed in auto accidents, suggests that alcohol may be a greater factor in such accidents than statistics indicate, and shows the importance of a

nation-wide survey of the relationship of alcohol to automobile accidents.

The chemical test for alcohol in body fluids will be an important factor in arriving at conclusions concerning the intoxicating ability of certain beverages.

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THE PATHOGENESIS OF NEUTROPENIA

A THEORETICAL CONSIDERATION*

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A decrease or absence of neutrophilic leukocytes in the blood stream has been a subject of growing importance during the past thirty-two years. However, until 1922, neutropenia had been recognized only as a part of certain well known clinical syndromes. Then it was recognized by Schultz as a rapidly fatal symptom complex which included marked prostration, peripheral neutropenia, and a severe grade of progressive oral sepsis. In the past eleven years, much additional information has been gained, but the etiology still remains a baffling problem. The rapidly increasing literature has brought out two important observations which have tended to eliminate the most widely accepted hypothesis of pathogenesis, that is, that the neutropenia is secondary to a septic or toxic process which causes an aplasia of the myeloid tissue of the bone marrow. The first observation shows that the neutropenia may definitely precede the appearance of infection.^{22, 25, 9, 4} The second observation demonstrates that myeloid aplasia does not always accompany neutropenia. It is now suggested that the condition of the myeloid tissue may not be the primary pathologic mechanism of this disease.

In approaching the consideration of an hypothesis to attempt to explain the mechanism of neutropenia, it will be necessary to consider briefly the pathology and physiology of the myeloid tissue in reference to the neutropenic state, and to think of the myeloid tissue as constituting an organ with all the potentialities for hypertrophy, atrophy, and functional insufficiency that appertain to any other organ.

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The use of a simple diagram (fig. 1) will assist in making the discussion more intelligible. The myeloid tissue of the bone marrow is represented by a white cone shaped area. The line A, drawn across the cone, indicates the normal level at which the myeloid tissue functions, which will be represented as ++. The area to the right will represent the blood stream in which is a neutropenia.

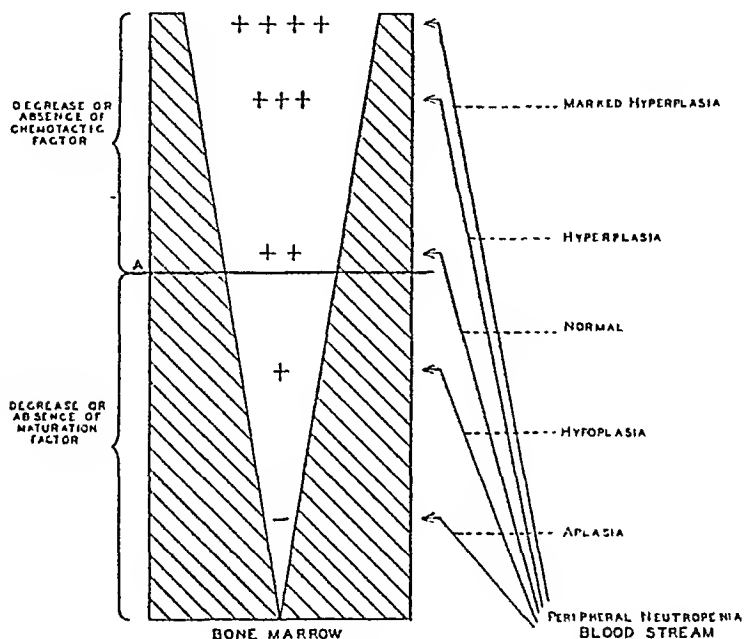


FIG. 1

When one finds the neutrophilic cells greatly reduced in the blood stream, that is, when there is a peripheral neutropenia, the first question which comes to mind is; what is the pathology in the bone marrow where these cells are formed?

When these cases were first being reported and autopsies obtained, the assumption that myeloid aplasia was the pathology underlying this disease seemed to be substantiated, as that was the condition found.^{22, 20, 14, 1, 23} This "aplasia concept" had developed partly on this basis of studies of bone marrow taken at autopsy, and partly on the basis of analogy to established findings in certain other conditions, such as in benzol poisoning, et cetera.

Necropsies on all of those patients having peripheral neutropenia disclosed myeloid aplasia, and until 1929, this question seemed to be settled. I will represent this myeloid aplasia by a minus sign at the bottom of the cone.

In 1929, Buck⁶ published the report of a patient who had peripheral neutropenia while biopsy of the sternal marrow showed normal myeloid tissue. The necropsy diagnosis on the bone-marrow was "acute hematopoiesis." This same case was included with a series reported by Dameshek and Ingall.⁸ Baldridge and Needles,³ reported a case which had lasted over a period of about four years. They finally resorted to splenectomy as a possible cure. There was not the usual rise of leukocytes after the operation as in other cases, but there was the usual platelet response. The patient died thirty-five days after the operation. The bone-marrow at autopsy disclosed an overgrowth of myelocytes and myeloblasts almost as marked as in myelogenous leukemia. This added to the records another case with peripheral neutropenia and a marked myeloid hyperplasia. However, this result was considered unsatisfactory because a biopsy of the bone-marrow was not obtained before splenectomy, and it was felt that the splenectomy may have played some part in the myeloid hyperplasia. The true status of these findings in fatal cases was substantiated later, however, when Fitz-Hugh and Krumbhaar¹⁵ reported three cases of neutropenia, one of which showed a marked myeloid hyperplasia at necropsy. The other cases were said not to be aplastic. The fact was then established that marked myeloid hyperplasia could underlie peripheral neutropenia. This marked myeloid hyperplasia is represented in the diagram by the + + + + signs.

Rosenthal²⁴ reported data on a group of patients having benign neutropenia (patients who recovered) on two of whom biopsies of sternal marrow were made. In these patients he found normal or hyperplastic myeloid tissue. This finding added to our group cases of recovery from peripheral neutropenia with normal or moderately hyperplastic myeloid tissue. This moderately hyperplastic myeloid tissue is represented in the diagram by + + +.

There are other cases reported in which the patients seem to

have normally a leukopenia and neutropenia,²⁴ and some patients who have what is called a fifty-fifty differential count; for example 45 per cent neutrophils and 45 per cent lymphocytes. The myeloid tissue, in these patients, is evidently functioning at a lower level, although I do not have biopsies on bone-marrow to substantiate this. Roberts and Kracke²² stated "These are the patients who are most likely to develop a severe neutropenia." I represent this type of myeloid tissue in the diagram by +.

The fact is now established that with peripheral neutropenia there can be almost any type of myeloid tissue from aplastic to markedly hyperplastic. To summarize: deaths have occurred with peripheral neutropenia and myeloid tissue of aplastic to markedly hyperplastic nature; recoveries have occurred with peripheral neutropenia and normal or hyperplastic myeloid tissue. The only condition not reported is recovery with a marked hypoplastic or aplastic marrow.

What is it that keeps the myeloid tissue functioning at a normal level, keeping the production of neutrophilic cells at a constant pace with their continuous destruction? There are two factors which function in the reproduction, growth, and delivery of the myeloid cells. The first, a maturation factor, causes the original stem cells to multiply and grow to maturity. This may be compared to the hormone which causes the development and ripening of the graafian follicles. When the original stem cells have grown to maturity and the myeloid tissue is formed, the stimulation for further growth is determined by functional demand. This demand in most organs and tissue is brought about by the wearing out of cells; as for example the wearing off of the surface epithelium which constantly stimulates the malpighian layer to activity. This demand, in the case of the myeloid tissue, is furnished in the form of a chemotactic factor which causes the neutrophilic cells to be delivered to the blood stream. As the neutrophilic cells are called to the blood stream, the normal stimulus is to replace them. This normal replacement continues as long as the maturation factor is present, even in long sustained leukocytoses.

Pyogenic organisms exert a chemotactic effect on the myeloid

tissue. All are familiar with the marked sustained leukocytosis in many pyogenic infections. In these cases, the generation of new myelocytes occurs simultaneously with the increased delivery of mature neutrophils, and the bone-marrow represents a "shift to the left" in the cell phases and an extension of myeloid foci proportionate to the need as long as it exists. This concept of leukocytosis postulates the pyogenic organisms as introducing a chemotactic factor which calls out the cells thereby producing a need for greater production. However, the exact way in which this chemotactic effect is produced is not known. Bacon et al.,² consider that this activity of the marrow comes from altered body proteins. It hardly seems likely that these pyogenic organisms introduce this chemotactic factor, but that they introduce a substance which stimulates the body tissues to produce this factor in excess. Some patients having neutropenia have shown a normal neutrophilic response when an infection developed. It is suggested that the organisms stimulated the body tissues to produce the chemotactic factor.

Some organisms exert this chemotactic effect, and at the same time introduce a myeloid or maturation depressant factor. Influenza and typhoid bacilli are examples. The myeloid tissue does not respond with a sustained leukocytosis in these infections, but instead there is a leukopenia. Doan et al.,¹¹ injected large doses of inactivated typhoid bacilli into animals, and found it was possible to call the neutrophilic cells from the marrow, producing an aplasia of the myeloid tissue without the corresponding activity to replace them. That inactivated typhoid bacilli may have this same depressing effect on the myeloid tissue in susceptible individuals, is indicated by two cases of fatal neutropenia following typhoid vaccination.^{5, 21}

The chemotactic effect of nucleic acid and its degradation products (pentose nucleotide, guanine, adenine, et cetera), on the myeloid tissue has been demonstrated by many experiments.^{10, 13, 17} It has been shown that the neutrophilic cells, when breaking down in the circulating blood, liberate these products. The theory is that these liberated products in turn stimulate the myeloid tissue to produce more cells.^{12, 13, 17, 26} In neutropenia

from any cause, these products would be greatly diminished in the blood stream. Out of this experimental work has come a therapeutic product, nucleotide K-96, made from the nucleic acid of yeast.¹⁸ Doan's¹⁰ experimental work on normal rabbits with nucleotide K-96 would lead to the assumption that it supplies a maturation factor, as well as a chemotactic factor, since, after repeated large doses given to normal rabbits, there was a marked myeloid hyperplasia of the bone-marrow with myeloid deposits in the kidneys and spleen. This can be interpreted as a replacement reaction. When cells are called out from normal marrow, the normal reaction is to replace them. If they are called out in excess, the tendency is toward an over production. In proof of the above interpretation, are the experiments of Harkin¹⁶ who produced neutropenia in rabbits with benzol, and found that pentose nucleotide did not have this effect on the myeloid tissue.

Unfortunately there is much less to be said concerning maturation factors. Our exact knowledge is confined to a maturation factor for erythrocytes. This is supplied in the liver extract and other tissue extracts used in the treatment of pernicious anemia. In pernicious anemia, the erythrocytes cease to multiply and develop properly, development ceasing at the megaloblastic stage, and the erythrocytes in the peripheral circulation are greatly reduced. Liver extract causes the function of normal reproduction to return, which is evidenced by a reticulocyte rise promptly after its use, and the erythropoietic tissue gradually returns to normal. May not malignant neutropenia follow the same course? There are remissions in both pernicious anemia and neutropenia. Perhaps what appear to be fulminating cases of malignant neutropenia with aplastic bone marrow have had many undetected attacks and remissions. These observations point to the fact that there must also be such a growth stimulating substance for the cells of the myeloid tissue. This substance is probably in a remote organ or tissue, as is the maturation factor for erythrocytes. It is not in the spleen, as this function continues normally after splenectomy. The liver seems a likely place for the manufacture of this factor, since this organ functions both as the erythropoietic and granulopoietic organ in early foetal life. The

liver extract now in use does not contain this factor. No doubt there is a specific maturation factor for each stem cell, and it may only require a different process of extraction than that now used.

Based on the theories so far advanced, and the experimental work cited to support them, we may formulate a theory of pathogenesis. Briefly the theory is one of pathological physiology.

Returning to the diagram, we may draw a bracket to embrace the myeloid tissue from the normal level, to and including the hyperplastic. Cases of neutropenia with this pathology can be considered as being due to a decrease or absence of the chemotactic factor. When the chemotactic factor ceases to function, maturation will continue, but the cells will not come to the circulation. This produces a normal and perhaps a hyperplastic myeloid tissue. Another bracket may be drawn embracing the hypoplastic and aplastic marrow. Cases of neutropenia with this pathology can be considered as being due to a decrease or absence of the maturation factor. If the maturation factor ceases to function, the chemotactic factor will continue to call the cells out until an aplasia is produced.

The absence of the chemotactic factor may be just as serious as the absence of a maturation factor, as both result in peripheral neutropenia, and peripheral neutropenia existing for any great length of time, leads to many serious complications and death.

All of the fatal cases so far reported have shown aplastic myeloid tissue, except the five reviewed in this paper. A chemotactic factor was not given to these patients, as nucleotide K-96 was not available at that time. Since the myeloid tissue was hyperplastic, they might have recovered with nucleotide therapy, if it could have been administered before infection was too far advanced.

Patients who recover spontaneously may lack one or the other of these factors, the function resuming without an outside aid, thereby producing a remission.

Biopsies of the bone marrow of Rosenthal's patients who later recovered, indicate that the pathology could have been due to a lack of the chemotactic factor, as the bone marrow was normal or hyperplastic.

There is a cyclic case on record, case 3 of a series reported by

Doan.⁹ A neutropenia of an alarming degree developed every twenty-one to twenty-three days. This neutropenia was not influenced by nucleotide K-96, which would tend to indicate that a chemotactic factor was not needed. A report was not made of biopsy of bone-marrow. However, this lack of reaction to nucleotide K-96 would lead to the assumption that the myeloid tissue became markedly hypoplastic every twenty-one days. It would seem that in this patient, the production of the maturation factor functioned in cycles, every twenty-one days ceasing to function for a few days, then resuming functioning.

Jackson et al.,¹⁸ treated a series of thirteen cases of neutropenia with nucleotide K-96. Five of the patients, with no essential clinical differences from those in the recovered cases, died in spite of active nucleotide therapy. Clinically the cases were the same, but evidently the underlying pathology was different. In a larger series, reported at a later date by the same authors,¹⁹ there is practically the same percentage of patients who did not respond to active nucleotide therapy. Does it not seem reasonable to suppose that the recovered cases had normal or hyperplastic myeloid tissue, and lacked a chemotactic factor which was supplied by the nucleotide K-96, while the fatal cases had aplastic myeloid tissue and lacked a maturation factor which could not be supplied? If the myeloid tissue is aplastic, a substance to call the cells to the periphery would be of little value. I believe this same cause was operative in my failure, and the failure of others, to obtain a response of the neutrophils in cases of aplastic anemia and leukanemia treated with nucleotide K-96.

Taussig and Schnoebelin,²⁷ in a review of 328 cases (which included the so-called secondary types) found that 25 per cent recovered without special therapy and with miscellaneous forms of treatment; 37 per cent recovered with blood transfusion, and 47 per cent with roentgen ray treatment. Nucleotide K-96 has raised the percentage of cures to seventy-four, as the 27 per cent of cases needing a chemotactic factor are now being adequately treated. The 26 per cent now dying in spite of all available treatments may constitute the aplastic, or markedly hypoplastic group, needing a maturation factor.

It is, of course, not contended that anything approximating definite proof of a maturation and chemotactic factor in this disease has been adduced. It does seem reasonable to deduct from the information at hand, that the pathology in the myeloid tissue does not constitute the primary pathologic mechanism of this disease.

This material has been presented in this way to stimulate interest in attacking this problem from a different angle; that is, to search for an organ or tissue extract which will supply a maturation factor for neutrophils. There should be a further search for active principles along the lines of the chemical investigations of Cohn et. al., and West and Howe,²⁸ a new type of hematological research, in which the goal is to find the chemical substance operative at each stage in the normal processes of division, growth and maturation of neutrophils. Such a therapeutic agent would aid the cases having hypoplastic and aplastic myeloid tissue, as well as prevent the occurrence of fulminating attacks in the chronic neutropenic patients.

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THE DIFFERENTIATION AND STANDARDIZATION OF CERTAIN STREPTOCOCCUS TOXINS AND ANTITOXINS BY MEANS OF THE SKIN TEST*

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Within the last few years, both in experimental investigations and in practice, the skin test has assumed an important position in the diagnosis of various pathological processes and susceptibilities to many types of infection. Among the earliest uses may be mentioned the Von Pirquet (1907) and the Mantoux (1908) tests in tuberculosis, followed by the Schick Test (1913) for the diagnosis of susceptibility to diphtheria. Skin tests have been used experimentally by Birkhaug (1924) in connection with the description of the hemolytic streptococcus of erysipelas; by Giordano (1929) for the diagnosis of undulant fever; by Ferry, Norton, and Steele, (1931) in experimental studies on meningococcus toxin; and by Thomas and Touart (1932) in conducting a clinical investigation as to the specificity and antigenic response of bacterial antigens. For many years skin tests have been subjected to practical clinical use in the diagnosis of various allergic conditions. These afford but a few illustrations of the scope of the intradermal test.

In 1924, Doctors George F. and Gladys H. Dick announced the results of skin tests with filtrates of blood broth cultures of hemolytic streptococci. This led to the discovery of scarlet fever streptococcus toxin and to the development of the Dick test, now an accepted clinical procedure. For use in the Dick test, scarlet fever streptococcus toxin is standardized as to the number of skin test doses per cubic centimeter, then diluted so that 0.1 cc. contains exactly one skin test dose. This is injected intradermally and the reading is made at the end of twenty-four

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hours. A positive reaction is observed if an area of erythema of 1 cm. or more in diameter surrounds the point of injection. Degrees in susceptibility are determined by the size of the area of erythema as well as by the intensity of the reaction. Through the use of a standardized scarlet fever streptococcus toxin, the official unit of scarlet fever streptococcus antitoxin may be determined. Thus, the unit is defined "in terms of the power to neutralize skin test doses of toxin."

Birkhaug² reported that the appearance of positive reaction following the intradermal injection of erysipelas streptococcus toxin is specific and indicates susceptibility to erysipelas. This view has not been shared by Williams,¹¹ Kirkbride and Wheeler,⁶ and others, although Thompson and Thompson⁹ in their elaborate review on streptococci state that there can be little doubt that the hemolytic streptococcus of erysipelas "is distinctly different" from that of scarlet fever. Dick and Dick⁴ reported the results of studies on specificity of soluble toxins produced by hemolytic streptococci, including the study of twenty-five strains each of hemolytic streptococci isolated from cases of scarlet fever and erysipelas. They called attention to errors in the results of skin tests which may emanate from the use of toxins of insufficient strength, from local tissue immunity, and from sensitivity to serum protein. It was shown by the results of this work that "the soluble toxins produced by scarlet fever and by erysipelas hemolytic streptococci are immunologically specific and distinct." The results of practical clinical use of erysipelas streptococcus antitoxin tend to confirm this conclusion.

It is generally conceded that hemolytic streptococci are frequently associated with puerperal septicemia. Lash and Kaplan⁷ followed the work of the Dicks on the hemolytic streptococcus of scarlet fever by publishing the results of their work on a blood broth filtrate of hemolytic streptococcus derived from puerperal septicemia. In subsequent reports, these investigators announced that a true specific toxin was derived from strains of puerperal septicemia streptococci from which antitoxin was produced. The results of skin tests on the same patients with toxins from both scarlet fever and puerperal septicemia strepto-

cocci served to confirm their findings. Williams confirmed the findings of Burt-White,³ Joe,⁵ and Stent,⁸ that positive or negative Dick tests were unrelated to pregnancy or to liability to puerperal sepsis. Thompson and Thompson¹⁰ in consideration of the outstanding literature conclude that the majority of severe cases of puerperal septicemia are caused by hemolytic streptococci, but that researches "on toxic filtrates of hemolytic streptococci, from puerperal cases, have so far yielded very little confirmatory evidence in favor of specificity."

Andrewes and Christie¹ found that the most refined methods of agglutination failed to differentiate between some strains of hemolytic streptococci found in scarlet fever, erysipelas, puerperal septicemia. They concluded that perhaps the important criterion in identifying these organisms depended upon the degree of intensity or potency of specific toxins which they were able to produce. Therefore, it is important that reliable data be accumulated bearing upon the specificity of the toxins from the scarlet fever and erysipelas hemolytic streptococci, and especially upon that derived from hemolytic streptococci found in puerperal septicemia.

PRESENT INVESTIGATION

During the last few years, an opportunity has been afforded the writers to study the results of skin tests involving the use of a relatively large number of samples of toxins and antitoxins derived from the scarlet fever and erysipelas hemolytic streptococci, and hemolytic streptococci isolated from severe cases of puerperal sepsis.

Since 1928, 6959 human subjects, including those tested for susceptibility, have been employed, involving 27,713 individual intradermal injections, in determining the potencies of many different lots of the respective toxins and antitoxins. Records for the past four years include the following: scarlet fever streptococcus toxins and antitoxins, 2390 susceptible human subjects, involving 15,528 individual intradermal injections; erysipelas streptococcus toxins and antitoxins, 336 susceptible human subjects, involving 1654 individual tests; puerperal septi-

cemia streptococcus toxins and antitoxins, 160 susceptible human subjects, involving 811 individual tests. The above total number of susceptible human subjects does not refer to individual but rather to test subjects. In many cases the same individuals were subjected to repeated tests.

This experience serves as a background for the present investigation, the purpose of which is (1) to show the significance of cross reactions among human susceptibles to the toxins of scarlet fever, erysipelas, and puerperal septicemia hemolytic streptococci, respectively; (2) to show the degree of specificity of the respective antitoxins toward the homologous toxins.

TOXINS EMPLOYED, TECHNIQUE, AND NATURE OF TESTS

Government standard scarlet fever streptococcus toxin has been used throughout this work. No officially recognized standardized toxin exists with respect to the erysipelas hemolytic streptococcus or to hemolytic streptococci obtained from puerperal septicemia. As has been pointed out by the Dicks, much confusion has resulted in work previously reported because of the lack of standardization of toxins used in conducting skin tests. They found that the filtrate from erysipelas strains contains considerably weaker toxin than that from the scarlet fever streptococcus. It is observed that the same condition obtains in toxins derived from the hemolytic streptococci found in puerperal septicemia. Relatively few strains are found which will produce toxin possessing a potency of more than 2500 skin test doses per cubic centimeter. No toxins should be employed containing less than 2500 skin test doses per cubic centimeter, because if the final dilution in the neutralization test is less than 1:250, non-specific protein reactions from the culture medium may be mistaken for positive skin reactions. The government standard scarlet fever streptococcus toxin which has been used throughout contains 4500 skin test doses per cubic centimeter. The erysipelas streptococcus toxin used in the early part of this work was derived from three strains, one of which was received from Dr. Sanford, Mayo Clinic, and two of which were isolated from blood cultures at the Henry Ford and Herman Kiefer Hospitals,

TABLE 1

Scarlet fever streptococcus antitoxin.....	90 lots
Scarlet fever streptococcus antitoxin.....	884 horse serum samples
Scarlet fever streptococcus toxin.....	81 lots
Erysipelas streptococcus antitoxin.....	49 lots
Erysipelas streptococcus antitoxin.....	82 horse serum samples
Erysipelas streptococcus toxin.....	24 lots
Puerperal septicemia streptococcus antitoxin.....	19 lots
Puerperal septicemia streptococcus antitoxin.....	51 horse serum samples
Puerperal septicemia streptococcus toxin.....	17 lots

TABLE 2

ERYSIPELAS STREPTOCOCCUS ANTITOXIN (SERUM SAMPLES)

SUB- JECT	READ- ING	TEST* TOXIN 1 S.T.D.	SERUM† SAMPLE 3025	SERUM SAMPLE 3027	SERUM SAMPLE 3035	SERUM SAMPLE 3101	SERUM SAMPLE 3159	SERUM CONTROL
1	hours 24	25x30 R†	12x13 R	9x 9 R	9x 9 R	12x11 R	7x 7 R	Neg.
	48	25x30 FR	12x12 R	9x11 R	12x12 R	11x12 R	11x11 R	Neg.
2	24	Serum reaction						
	48	Serum reaction						
3	24	20x20 R	10x11 R	9x 8 R	9x 9 R	11x11 R	7x 7 R	Neg.
	48	20x20 FR	11x11 R	11x11 R	11x15 R	11x12 R	8x 9 R	Neg.
4	24	25x20 R	12x11 R	11x11 R	12x12 R	11x12 R	12x12 R	Neg.
	48	20x20 R	12x11 R	11x11 R	12x12 R	11x12 R	8x 9 R	Neg.
5	24	25x25 R	12x12 R	10x10 R	9x 9 R	11x12 R	8x 9 R	Neg.
	48	25x25 R	12x11 R	10x10 R	13x13 R	12x13 R	11x12 R	Neg.
Values§.....			3 plus	3 plus	2 plus	3 plus	3 plus	

* One-tenth cubic centimeter (1:300 dilution) or one skin test dose toxin.

† Serum samples from individual horses under immunizing treatment mixed with one skin test dose toxin.

‡ R = red. FR = faint red.

§ Determined on 48-hour readings as follows: 4 plus strong = complete neutralization at 100 units. 4 plus = neutralization (area of erythema less than 1.0 cm. in all subjects). 3 plus = reaction from 1.0 cm. to 1.3 cm. 2 plus = reaction from 1.3 cm. to 1.5 cm. 1 plus = reaction from 1.5 cm. to 1.8 cm. N.G. = reaction above 1.8 cm.

Detroit. This toxin has been found to contain 3000 skin test doses per cubic centimeter, the dilution of the toxin used as the

standard in the tests being 1:300. The original test toxin derived from the bouillon filtrates of streptococci from puerperal septicemia cases represented three strains obtained from blood cultures. The toxin resulting from the growth of these cultures has been found to contain 4000 skin test doses per cubic centimeter, requiring for 0.1 cc. intradermal injection a dilution of 1:400. A new test toxin has been prepared from the same strains grown under different conditions, which produces more distinct reactions and probably contains slightly more than

TABLE 3
PUERPERAL SEPTICEMIA ANTITOXIN (SERUM SAMPLES)

SUBJECT	READING	TEST TOXIN 1 S.T.D.	SERUM SAMPLE 3092	SERUM SAMPLE 3113	SERUM SAMPLE 3114	SERUM CONTROL
1	<i>hours</i>					
	24	20x23 R	Neg.	10x13 R	10x10 R	Neg.
	48	20x23 FR	Neg.	12x13 R	11x12 R	Neg.
2	24	20x20 R	Serum reaction			
	48	20x22 R	Serum reaction			
3	24	18x18 R	10x10 R	11x11 R	10x11 R	Neg.
	48	18x18 R	10x 9 R	12x12 R	10x10 R	Neg.
4	24		Unable to obtain reading			
	48	25x30 R	7x 7 R	13x14 R	11x11 R	Neg.
5	24		Unable to obtain reading			
	48	18x20 R	7x 8 R	13x14 R	11x11 R	Neg.
Values.....			4 plus	2 plus	3 plus	

4000 skin test doses per cubic centimeter. The above toxins have been used in the routine testing of the lots of materials listed in table 1.

The technique employed has been that which is followed routinely in observing the requirements of the National Institute of Health and of the Scarlet Fever Committee with respect to the standardization of scarlet fever streptococcus antitoxins and toxins.

Protocols are submitted as illustrative of the nature of the

intradermal tests on various lots of serum samples of erysipelas, and puerperal septicemia streptococcus antitoxins in tables 2 and 3.

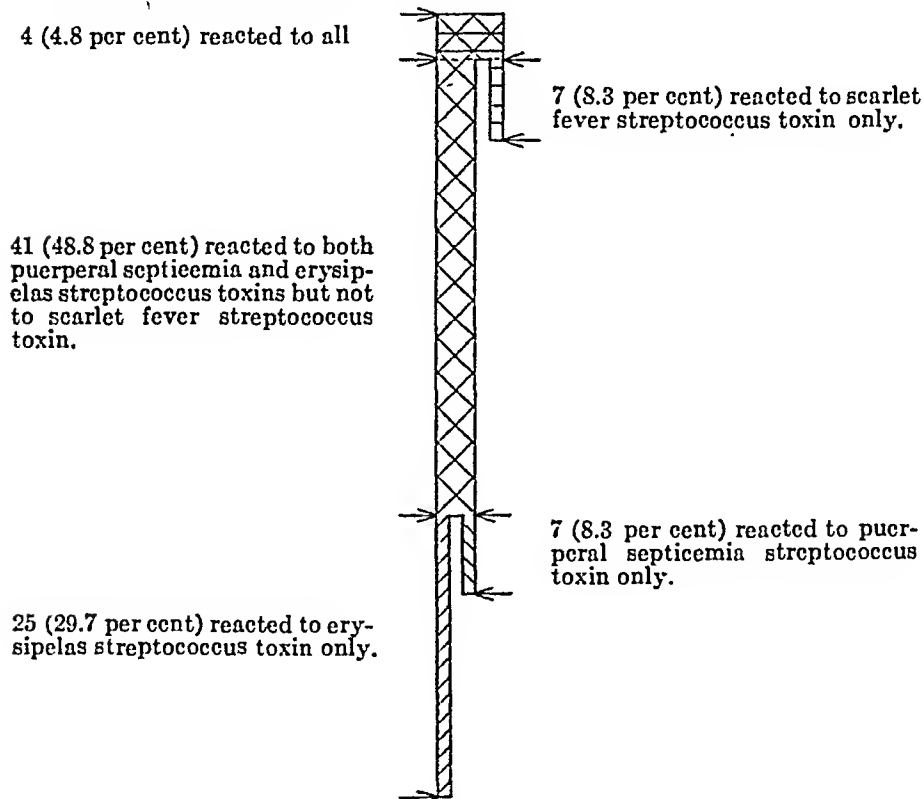
CHART 1

CROSS REACTIONS

Out of 221 individuals tested for susceptibility to scarlet fever, puerperal septicemia and erysipelas streptococcus toxins,

137 (62 per cent) reacted to none	84 (38 per cent) reacted to one or more
-----------------------------------	---

The 84 reactors were distributed as follows:



EXPERIMENTAL DATA

In order to determine the extent of cross reactions which might be observed among human subjects susceptible to one or more of the three toxins under consideration, a group of 221 subjects was

selected who were tested each with one skin test dose intradermally of the three toxins. One hundred and thirty-seven or sixty-two per cent reacted to none of the three toxins, while eighty-four, or 38 per cent, reacted to one or more. Of the total original 221 subjects, positive reactions were observed as follows: to scarlet fever, eleven, or 5 per cent; to erysipelas, seventy, or 31.6 per cent; to puerperal septicemia, fifty-three, or 23.5 per cent. Of the eighty-four reactors, four (4.8 per cent) individuals reacted to all three toxins; seven (8.3 per cent) to scarlet fever streptococcus toxin only; twenty-five (29.7 per cent) to erysipelas streptococcus toxin only; seven (8.3 per cent) to puerperal septicemia streptococcus toxin only; and forty-one (48.8 per cent) to both erysipelas and puerperal septicemia streptococcus toxins. The results are shown in chart 1.

In the above series of reactors were found a certain proportion of individuals who were susceptible to only one of three toxins in each case. Of eighty-four reactors to one or more of three types of hemolytic streptococcus toxins, the following observations were made:

(1) Of the individuals positive to scarlet fever, 36.4 per cent were positive to erysipelas.

(2) Of the individuals positive to scarlet fever, 36.4 per cent were positive to puerperal septicemia.

(Those positive to erysipelas were also positive to puerperal septicemia and represent identical subjects, only four individuals being involved.)

(3) Of the individuals positive to erysipelas, 5.9 per cent were positive to scarlet fever.

(4) Of the individuals positive to erysipelas, 64.3 per cent were positive to puerperal septicemia.

(5) Of the individuals positive to puerperal septicemia, 7.7 per cent were positive to scarlet fever.

(6) Of the individuals positive to puerperal septicemia, 86.5 per cent were positive to erysipelas.

No evidence is observed which shows any direct interrelation between the toxins of the three hemolytic streptococci.

In order to study further the possibility of cross neutralization

TABLE 4

SPECIFICITY OF PUERPERAL SEPTICEMIA STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF PUERPERAL SEPTICEMIA, ERYSIPELAS, AND SCARLET FEVER STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	TEST TOXIN PUERPERAL SEPTICEMIA 1:400 DILU- TION 0.1 CC.	PUERPERAL SEPTICEMIA TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	PUERPERAL SEPTICEMIA TOXIN PLUS ERYSIPELAS ANTITOXIN	PUERPERAL SEPTICEMIA TOXIN PLUS SCARLET FEVER ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
1	<i>hours</i> 24 48	No reaction No reaction					
2	24 48	15x18 R 15x17 R	Neg. Neg.	15x15 R 15x15 R	15x16 R 15x15 R	Neg. Neg.	Neg. Neg.
3	24 48	20x21 R 20x20 R	7x 6 R 10x11 R	20x20 R 20x18 R	20x19 R 19x19 R	Neg. Neg.	Neg. Neg.
4	24 48	15x20 R 18x23 R	Neg. Neg.	12x13 R 13x13 R	14x15 R 14x13 R	Neg. Neg.	Neg. Neg.
5	24 48	15x15 FR 15x15 FR	Neg. Neg.	14x14 R 16x15 R	14x14 R 16x16 R	Neg. Neg.	Neg. Neg.
6	24 48	15x17 R 15x15FR	Neg. Neg.	Neg. Neg.	Neg. Neg.	Neg. Neg.	Neg. Neg.

TABLE 5

SPECIFICITY OF ERYSIPELAS STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	TEST TOXIN ERYSIPELAS 1:300 DILU- TION 0.1 CC.	ERYSIPELAS TOXIN PLUS ERYSIPELAS ANTITOXIN	ERYSIPELAS TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	ERYSIPELAS TOXIN PLUS SCARLET FEVER ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
1	<i>hours</i> 24 48	17x20 R 17x22 R	Neg. Neg.	14x16 R 14x17 R	17x20 R 17x20 R	Neg. Neg.	Neg. Neg.
2	24 48	20x23 R 20x23 R	Serum reaction Serum reaction			25x29 R 25x30 R	Neg. Neg.
3	24 48	20x20 R 20x22 R	Serum and protein reaction Serum and protein reaction			Diffuse Diffuse	Diffuse Diffuse
4	24 48	22x25 R 22x27 R	7x7 R 10x12 R	20x22 R 22x25 R	20x25 R 23x27 R	Neg. Neg.	Neg. Neg.
5	24 48	24x20 R 22x22 R	Serum reaction Serum reaction			20x20 R 22x25 R	Neg. Neg.

and the possible inter-relationship between those reacting to the toxins of the scarlet fever, erysipelas, and puerperal septicemia streptococci, neutralization tests with the various antitoxic serums were conducted. Six human subjects, who had reacted positively to one skin test dose of puerperal septicemia streptococcus toxin, were tested with mixtures of the specific test toxin,

TABLE 6

SPECIFICITY OF SCARLET FEVER STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	GOVERNMENT STANDARD SCARLET FEVER TOXIN 1:4500 DILUTION 0.1 cc.	STANDARD SCARLET FEVER TOXIN 1:400 DILUTION PLUS SCARLET FEVER STANDARD ANTITOXIN	SCARLET FEVER TOXIN PLUS ERYSIPELAS ANTITOXIN	SCARLET FEVER TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
1	<i>hours</i> 24 48	20x22 R 20x22 R	Neg. Neg.	19x20 R 19x22 R	20x20 R 22x22 R	Neg. Neg.	Neg. Neg.
2	24 48	15x18 R 17x18 R	Serum reaction, diffuse Serum reaction, diffuse				Neg. Neg.
3	24 48	20x21 R 20x20 R	Neg. Neg.	20x21 R 19x21 R	20x23 R 20x23 R	Neg. Neg.	Neg. Neg.
4	24 48	No reaction No reaction					
5	24 48	17x16 R 17x16 FR	Neg. Neg.	14x16 R 15x17 R	14x14 R 16x17 R	Neg. Neg.	Neg. Neg.
6	24 48	15x15 R 17x15 R	Neg. Neg.	15x15 R 17x17 R	14x12 R 16x17 R	Neg. Neg.	Neg. Neg.

puerperal septicemia streptococcus antitoxin, erysipelas streptococcus antitoxin, and scarlet fever streptococcus antitoxin, respectively. Similar tests were conducted on five individuals susceptible to the toxin of the erysipelas streptococcus, and also on six positive Dick reactors. The results are shown in tables 4, 5 and 6.

Among six human subjects initially susceptible to the toxin of puerperal septicemia hemolytic streptococcus, one failed to react to the toxin. In the remaining five, complete neutralization of puerperal septicemia toxin by homologous antitoxin was observed in all but one, but no neutralization occurred with the antitoxins from scarlet fever and erysipelas streptococci. Likewise in the group of five previously found to react to erysipelas streptococcus toxin, two reactors showed satisfactory neutralization with homologous antitoxin, but failed to show neutralization to puerperal septicemia and scarlet fever streptococcus antitoxins, while none of four satisfactorily positive Dick reactors showed evidence of cross neutralization to erysipelas and puerperal septicemia antitoxins. It is recognized that at the present time scarlet fever streptococcus toxin is relatively stronger than either erysipelas or puerperal streptococcus toxins, which fact perhaps serves to explain the slight tendency toward cross neutralization in one instance in each series (erysipelas, and puerperal septicemia). The experimental evidence, however, definitely points toward specificity.

SUMMARY

The results of the above experimental work not only confirm the conclusions which were reached by the Dicks that "the soluble toxins produced by scarlet fever and erysipelas streptococci are immunologically specific and distinct" but suggest furthermore that the toxin produced by certain hemolytic streptococci isolated from cases of puerperal septicemia is specific and distinct.

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HISTOLOGY AFTER THORIUM DIOXIDE (THOROTRAST) IN HEPATOLIENOGRAPHY*

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The advent of a method of roentgenographic visualization of the liver and spleen in the human has opened a vast new field of scientific investigation. The procedure is based upon a physiologic principle of the reticulo-endothelial cells; that is, the characteristic property of engulfing colloidal particles which may be introduced into the circulation. That the method represents a definite step forward in hepatolienography has been confirmed many times. Yet, there has been considerable conjecture concerning many of the phases, as for example, the radioactivity of the metal; the hemoclastic activity of the colloidal suspensions; and the permanence of the thorium particles in the reticulo-endothelial cells with possible toxic effects produced after a long period of time.

Since the last clinical paper²⁵ published by myself and associates numerous results have been reported by various authors concerning different phases of the problem. We have continued our work, reporting ^{4, 24, 26} results from time to time. An attempt has been made to check these results with the work of other investigators; in each instance, sufficient experiments in a number of animals being performed in order to reduce to a minimum results which may be accidental. It is well known to those who do a great deal of work with animals that in isolated instances, many phenomena occur which are purely accidental or merely coincidental. Certainly, proper interpretation of results in the light of these facts is essential before any conclusions may be drawn.

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For a period of over two years, dogs, rabbits, guinea pigs, white mice and albino rats were injected with various dosages of thorotrast. The number totalled well over one hundred animals, with controls in every experiment. Later patients in whom it was deemed essential that further information regarding the liver and spleen be obtained for correct diagnosis were injected for diagnostic purposes. In all instances clinical data were noted in conjunction with clinicopathologic and histopathologic studies which were made at every opportunity.

It is the purpose of this paper to note these observations and attempt to correlate the findings with those of other investigators, in order to thoroughly understand the principles of the method, its possibilities, limitations and probable contraindications.

HISTORY

In 1929, M. Oka,¹⁵ while studying the metabolism of thorium dioxide in rabbits, noted that upon its administration only a fraction of the injected thorium was eliminated. He took radiographs of various parts of the body (thorium dioxide being a heavy metal, is opaque to the roentgen ray) and found shadows conforming in position to the spleen. He reported his finding, but did not explain the mechanism by which these organs could be made visible roentgenographically.

Immediately following Oka's publication, P. Radt¹⁸ and Jaffé⁸ began their work showing the thorium particles were engulfed from the circulation by the reticulo-endothelial cells, thus rendering the organs in which these particular cells happen to be present more opaque to the roentgen ray. The thorium dioxide used was marketed under the name of tordiol, which was toxic due to the low dispersion factor of the solution and flocculation properties when brought into contact with organic matter. Their work was repeated by Oka.¹⁶ Later Radt^{19, 20} succeeded in preparing the thorium dioxide in a so-called "stabilized state," in which it would not flocculate when brought into contact with body fluids and in increasing the degree of dispersion by relatively high concentrations of glucose solutions. This final substance is now marketed under the name of "thorotrast-Heyden."

The solution used contains 25 per cent thorium dioxide by volume suspended in a stabilized colloidal state. The element, thorium, is opaque to the roentgen rays, being a heavy metal (atomic weight, 232.15). On administration into the blood stream, the reticulo-endothelial cells engulf the particles of thorium dioxide. Since these cells are present in relatively large numbers in the spleen and liver, there is a difference in shadows cast by these organs and the surrounding tissues that contain few, if any, reticulo-endothelial cells. Whereas, before the introduction of this method, only faint rather indefinite outlines of the liver and spleen were obtainable by roentgenography, it is now possible not only to study the size and shape of these organs, but to determine more accurately whether any tumor, cyst or abscess is present in these organs or whether a certain tumor in the hepatic or splenic regions is within or outside the liver or splenic substances.

The question of dosage is of paramount importance. Kadrnka⁹ found that not more than 0.8 cc. of thorotrast per kilogram of body weight was required in the human to produce good contrast between the shadows cast by these organs and the surrounding soft tissue. Satisfactory radiographs of the liver and spleen have been obtained, using this dosage, in both our experimental animals and in the human.

The work was then taken up in this country by Yater and Otell,²⁸ Stewart, Einhorn and Illick²³ and my coworkers.²⁵

The reticulo-endothelial cells form the component parts or units of a system designated by Aschoff¹ as the "reticulo-endothelial system." The cells of this group are widely scattered throughout the body, being collected in relatively large numbers in the spleen, liver, medullary follicles and "cords" of lymph nodes. Considerable numbers are also found in the formative bone marrow, lung and in the adrenal and pituitary bodies. Maximow¹⁴ refers to the individual cells as "histiocytes" and cites sufficient reasons to bear out his contention for this nomenclature. These cells have the characteristic property of engulfing colloidal particles in fine granular form which may be present in the circulating body fluids. It is this definite functional

capacity which distinguishes this type of cell from the connective tissue elements and from all forms of myeloid and lymphatic cells. In this connection, however, it is well to recall that practically any type of cell may become phagocytic if suitably and sufficiently stimulated. Only those cells which have a native avidity for colloidal particles must be included in the reticulo-endothelial system. For instance, cells which become pigment-laden only after dye particles are injected in large amounts and in great concentration are not necessarily of the so-called reticulo-endothelial type.

The "endothelial" part of the term "reticulo-endothelial system" is derived from the fact that these cells form an interrupted layer lining many of the sinuses of several organs, namely, the liver and spleen. The question arises concerning the relationship of the reticular fibrils with the "reticulo" part of the term. Corner,³ confirming the work of Mall,¹³ has demonstrated the presence of extremely fine intracellular fibrils. These cytoplasmic projections branch out from the cells and form a fine reticular network in the intercellular spaces. As a result of this work, there exists another criterion, morphology, by which is distinguished the basic cells of the so-called "reticulo-endothelial system."

Numerous investigators have studied the problem in its various phases and from their studies results have been obtained and conclusions drawn, which are in many instances directly divergent. Radt^{18, 19, 20} and Kadrnka^{9, 10} after considerable experimentation in animals from a clinical and histopathologic standpoint obtained results from which they concluded that the method is most valuable as a clinical aid and quite harmless in the dosage used. Indeed, in certain of Kadrnka's cases, a definite beneficial therapeutic effect was obtained, although sufficient clinical data had not been accumulated to draw any sweeping conclusions. Otell¹⁷ after thoroughly reviewing the subject in both experimental animals and in the human, could not definitely demonstrate depression of the function of the cells comprising the reticulo-endothelial system. Yater and Otell²⁹ conclude from a study of about eighty cases that, "It (Thorotrast)

is apparently harmless and contraindications are negligible. Reactions are few and are not serious." Yater³⁰ has used thorotrast in one hundred human subjects with satisfaction. Lewisohn¹² following the utilization of thorotrast in four rabbits and six patients, concluded that, "the intravenous injection of Thorotrast in quantities mentioned has no immediate ill-effect on the patient." In this latter series the actual dosage was only noted in one case and the amount injected was 60 cc. (five doses of 12 cc. each). It is presumed that all patients received the same dosage.

The effect following intravenous thorotrast upon the immune mechanism has been studied by Held^{6, 7}. From the results obtained in his experiments with rabbits, he assumed that in the human, after the thorium dioxide particles have been completely stored by the reticulo-endothelial cells, no damage to this means of protection against infection need be feared. He also showed that only slight influence was exerted by thorotrast on the formation of antibodies; explaining his results by the fact that only a small part of the tissue concerned in the formation of antibodies stores the thorotrast.

However, other workers have reported results from which opposite conclusions have been drawn. Stewart, Einhorn and Illick²³ reported eight cases, including one in which a fatal hemorrhage occurred from a carcinoma of the stomach following thorotrast injections. They also found evidence of radioactivity in splenic tissue removed at autopsy from a patient following thorotrast injection. When the splenic tissue was placed in a petri dish in contact with a photographic plate, evidence of activity on the plate was noted after one day. Harris and Friedrichs⁵ working with white rats in which amounts of thorium dioxide varying from 0.2 cc. to 1 cc. was injected reported severe changes in the liver and spleen. Shih and Jung²¹ reported work with rabbits in which thorotrast was used in doses varying from 1.0 cc. to 9.0 cc. per kilogram. They reported,

All rabbits displayed at autopsy extensive extravasations of blood in various internal organs. It is concluded that thorotrast, probably due to its content of thorium dioxide given intravenously in rabbits, tends to lower the thrombocytic

content of the blood and to produce acute purpura hemorrhagica; amounts of three to four times the standard dose are usually fatal. The fact that the standard doses were given in two, rather than in four fractions as given by Kadrnka, may well account for the difference in our results and his.

Shute and Davis²² have recently reviewed the literature in a most complete manner. By using dosages of approximately 6 cc. to 7 cc. per kilogram of body weight in dogs and rabbits, they were not successful in attempts at roentgenographic visualization of the placenta of these pregnant animals as reported by previous investigators. They noted intense degeneration, particularly in the liver and spleen. One of their rabbits aborted and a spontaneous rupture of the spleen with hemorrhage was found in this animal.

Whitaker, Davie and Margatroyd²⁷ recently tabulated the advantages and disadvantages of the method and described the histologic and histopathologic pictures in the spleen, bone marrow, lung, kidney and suprarenal gland of rabbits. The rabbits were given one or two doses of thorotrast. The dosage was roughly proportionate to the maximum of that recommended for human beings.

EXPERIMENTAL METHODS

In the light of this maze of conflicting experimental and clinical results, sufficient experiments were performed in various animals using dosages of thorotrast varying from that which produced satisfactory roentgenographic visualization of the liver and spleen (0.8 cc. per kilogram of body weight), to ten times this dosage (8.0 cc. per kilogram of body weight). The complete dose was divided into three fractions, each being given twenty-four hours apart. Observations of the internal organs were made at varying times by means of laparotomies and postmortem examinations. Practically all tissues of the body were taken at autopsy and sections of the spleen and liver taken at operation. The time at which the sections were taken following thorotrast injection varied from twenty-four hours to two years and two months.

Eighty-six animals were used in this work, including five dogs, fifteen albino rats, eighteen guinea pigs, twenty-one white mice

and twenty-seven rabbits. An additional number of animals were used as controls in each experiment. In addition, histologic studies were made at necropsy of the patients who were injected for diagnostic purposes.

A. Animals receiving 0.8 cc. per kilogram of body weight divided into three doses, each dose given twenty-four hours apart

1. *Liver.* At the end of twenty-four hours a few thorium particles were still found free in the capillaries and in the liver cells. However, the greater portion

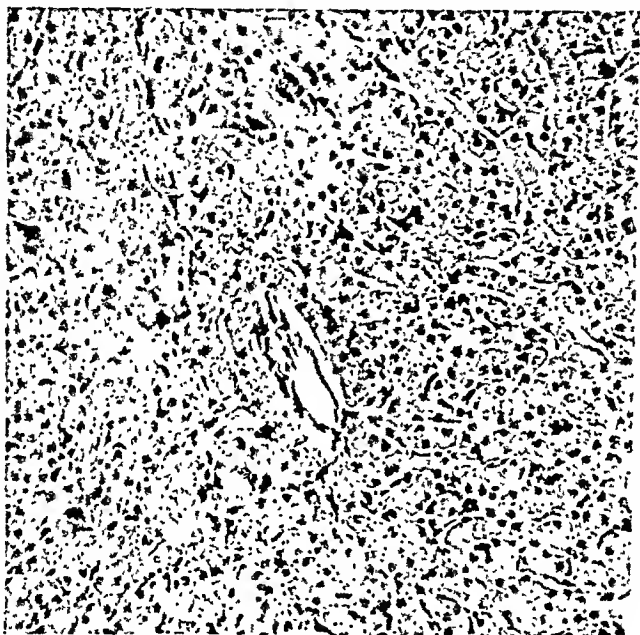


FIG. 1. LIVER OF DOG

One month following 0.8 cc. thorotrast per kilogram body weight. Reticulo-endothelial cells filled with thorium dioxide particles. Liver cells do not show any remarkable changes. $\times 350$

had already been engulfed by the reticulo-endothelial cells, which had become considerably larger in size. At the end of one week no free thorium was found, but the reticulo-endothelial cells had become swollen and the nuclei had begun to take a position near the periphery of the cell. Only an occasional thorium granule was present in the liver cells. One month after the injection of the thorium, no particles were seen in the liver cells, but a considerable amount was still present in the reticulo-endothelial cells diffusely throughout the organ (figs.

1 and 2). A few of the latter cells were noted in which the nuclei had divided and migrated to the periphery of the cell. Six months later practically the same picture was presented, except that here and there a few reticulo-endothelial cells not containing the thorium dioxide particles were found. At the end of two years and two months the thorium particles were still present in considerable amounts. The reticulo-endothelial cells containing the particles were no longer diffusely distributed throughout the organ, but had become aggregated in areas here and there. The thorium particles had apparently become considerably larger in size, entirely filling the cells. The nuclei of the reticulo-endothelial cells had divided and migrated to the periphery of the cell. These cells had

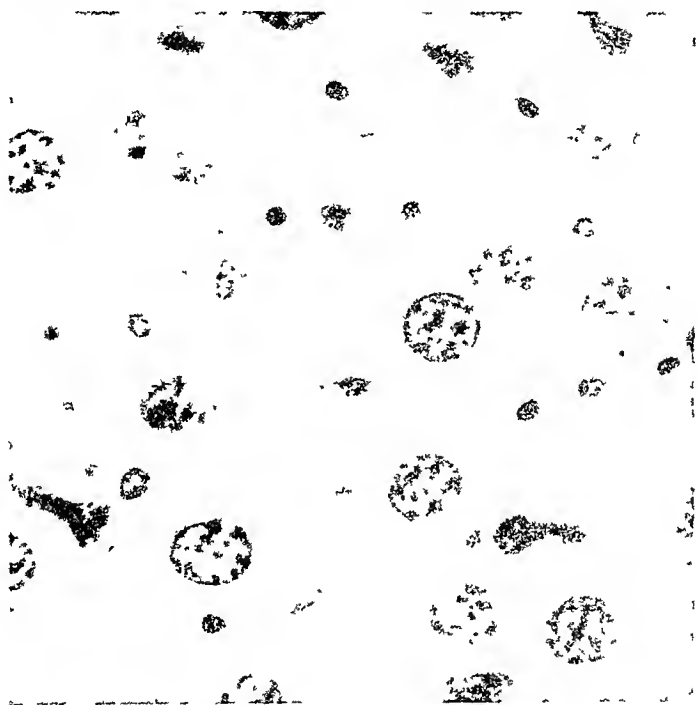


FIG. 2. SAME AS FIG. 1. $\times 500$

become as large or slightly larger than the liver cells and had aggregated in the sinuses as to almost completely fill their lumens. So packed were the thorium particles in the cells that the cytoplasm was no longer visible. Despite this accumulation of the thorium particles into some of the cells, other reticulo-endothelial cells showed no particles in them (fig. 8). Apparently, the reticulo-endothelial cells were dividing, the younger cells taking up the thorium particles which were liberated by the older cells which had succumbed. Those containing the thorium seemed to have a greater avidity for more particles, possibly due to the selectivity of different reticulo-endothelial cells for different substances. When one substance is present in a particular cell, it seems that as more of that

certain substance becomes available, the cell continues to engulf it. No evidence of hemorrhage or fibrosis could be determined in any of the sections studied.

2. *Spleen.* As regards the free particles of thorium and the reticulo-endothelial cells, the spleen presented essentially the same picture as seen in the liver at the various time intervals. In addition, there also appeared a few large giant cells, not of the multinucleated foreign body type, in tissue sections of

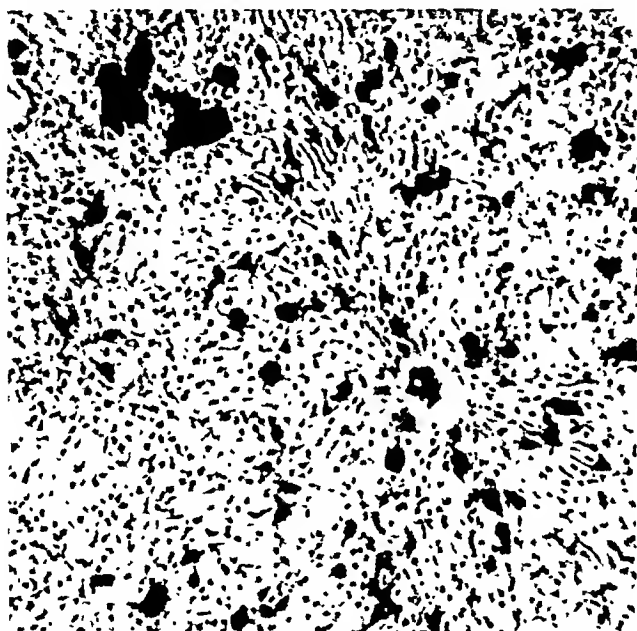


FIG. 3. LIVER OF RABBIT

One day following 8.0 cc. thorotrast per kilogram body weight. Thrombi of thorium particles in sinuses and extensive degeneration of liver tissue. $\times 350$

animals six months after injection. In the tissues removed at the end of two years, these cells were present in considerable numbers throughout the section. At no time did they manifest any phagocytic propensities (fig. 9). No evidence of hemorrhage or fibrosis or necrosis could be determined in any of the sections studied.

3. As a rule, none of the other organs revealed any evidence of thorium particles except the lungs. The later sections of lung showed the presence of a greater number of thorium-filled reticulo-endothelial cells than those tissues examined in the earlier periods. This was probably due to the migration of these cells to the lung, this organ being one of the routes of excretion.

B. Animals receiving 8.0 cc. thorotrast per kilogram of body weight divided into three doses, each dose being given twenty-four hours apart

1. *Liver.* Histological studies one day after injection revealed numerous thrombi present in the sinuses. On closer examination the reticulo-endothelial cells were literally loaded with the thorium granules and a great deal of thorium was found free in the sinuses and between the cells. A considerable number of thorium particles were found in the liver cells and occasionally a free thorium dioxide granule here and there in the bile ducts. There was an intense paren-

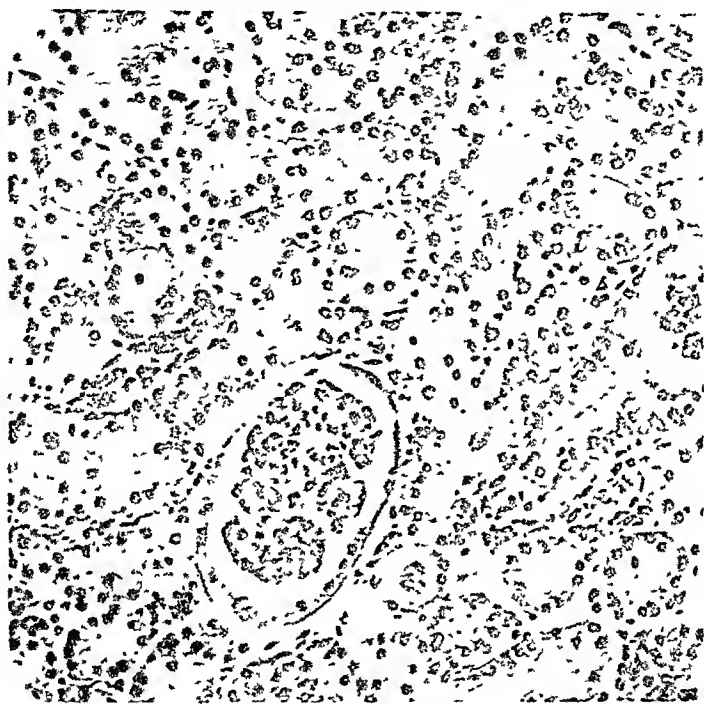


FIG. 4. KIDNEY OF GUINEA-PIG

One week following 8.0 cc. thorotrast per kilogram body weight. Glomerular capsule swollen and cloudy swelling of tubules present. $\times 400$

chymatous degeneration of the liver cells, many of which showed the nuclei to be pyknotic. Only slight, if any, actual necrosis of cell protoplasm was found (fig. 3). At the end of one week practically all of the thorium was seen within the reticulo-endothelial cells. There was a notable increase in the number of these cells. Coincidentally, many of the nuclei of these cells showed pyknosis and fragmentation. A few of the nuclei of the liver cells showed mitosis; probably evidence of regeneration of liver parenchyma. At the end of six months, much of the thorium had disappeared and the number of the reticulo-endothelial cells containing the thorium had become slightly lessened. However, the cells were markedly enlarged and formed thrombi in the venous sinuses. Some tho-

rium was found free, probably due to fragmentation of the reticulo-endothelial cells which had succumbed. The liver cells did not show any thorium particles present, but many of the nuclei had migrated to the periphery of the cell. No hemorrhage or fibrosis was noted in this organ.

2. *Spleen.* The changes seen here were similar to those observed in the liver at the various stages, there being innumerable thrombi of thorium particles both free and in the enormously enlarged reticulo-endothelial cells. Here and there, minute hemorrhages with beginning necrosis resulting from capillary



FIG. 5. LUNG OF WHITE RAT

One month following 8.0 cc. thorotrast per kilogram body weight. Reticulo-endothelial cells contain many thorium dioxide particles. $\times 100$

thrombosis was noted. At the end of one week, a most interesting histological picture from the standpoint of distribution of the reticulo-endothelial cells was observed in the spleens of the various animals. The spleens of the guinea pigs and rabbits revealed the thorium particles in the cells at the center of the Malpighian bodies, whereas the same organ in the white rats and mice presented the thorium particles in the cells at the periphery of the Malpighian body. At the end of one month and later, the only changes in the picture was a diminution in the amount of thorium present in the organ, the histological changes lasting throughout the period of observation.

3. *Kidneys.* Those animals sacrificed at the end of one day and one week

showed exudate with thickening of the glomerular capsule and thrombi of thorium in the capillary tufts. Considerable granular degeneration of the proximal convoluted tubules was found. However, at the end of one month and later, these changes were no longer found (fig. 4).

4. Thorium particles were found in the reticulo-endothelial cells of bone marrow (fig. 6), adrenal glands and a considerable amount in the lung (fig. 5). A few of the reticulo-endothelial cells filled with thorium were found in the lumen of the alveoli of the lung. No further notable changes in histological structure

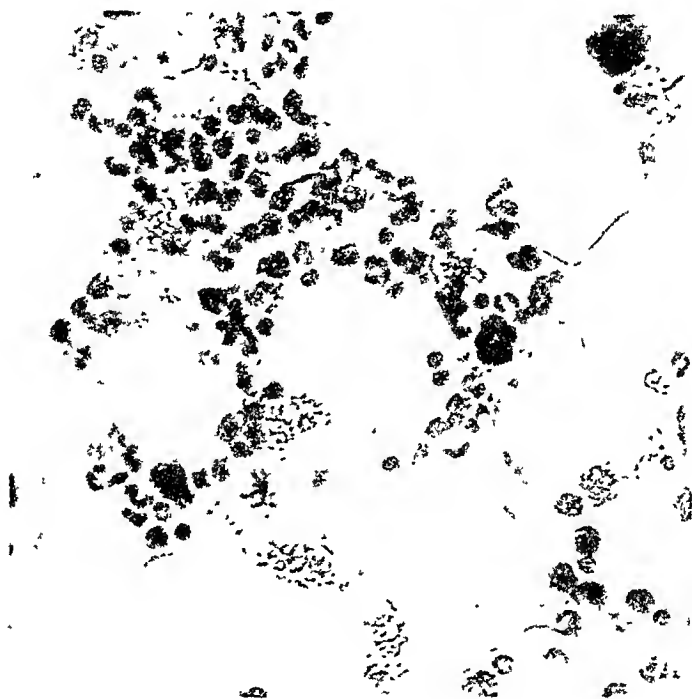


FIG. 6. BONE MARROW OF DOG

One week following 8.0 cc. thorotrast per kilogram body weight. Numerous reticulo-endothelial cells are seen containing thorium dioxide granules. $\times 400$

other than the presence of thorium particles were seen in any of these tissues studied.

5. Except in rare instances, no notable number of thorium particles were found in the sections examined from the brain, heart muscle, pancreas, genital organs, lymph nodes, or tissues of the embryos of the pregnant animals. Those animals wherein the intracardiac administration of the thorium was practised, accidental injection of the mediastinal tissues resulted in thorium particles being present in the pericardial and pleural sacs and also in the draining lymph nodes. Only a few of the animals were killed by punctures of the pleural sacs following laparotomy, while under the influence of chloralose, during experiments con-

cerning splenic contraction.⁴ The great majority were sacrificed by sudden dislocation of the skull from the vertebral column. All control animals were sacrificed by respective methods.

CLINICAL AND HISTOLOGICAL RESULTS IN HUMAN CASES

Approximately twenty-five patients in whom it was deemed essential that further information regarding the liver and spleen

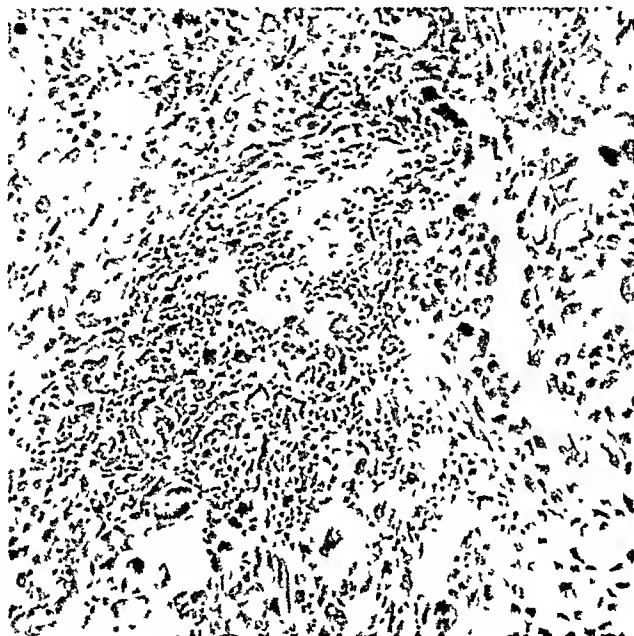


FIG. 7. LIVER, HUMAN

Forty-eight hours after 0.8 cc. thorotrast per kilogram body weight. Section at edge of metastatic carcinoma nodule. Carcinoma cells show no thorium dioxide granules. $\times 490$

be obtained before the proper therapy could be instituted were injected with thorotrast. Approximately 0.8 cc. of thorotrast per kilogram of body weight, divided into three doses, each given twenty-four hours apart, was injected in all cases. Twenty-four to forty-eight hours after the last injection, a plain antero-posterior radiograph was taken at a distance of 55 cm., using a potential of 90 K.V. and 50 milmps. for two seconds, with a Potter-Bucky diaphragm.

Clinically, little of note occurred at the time of injection which could be considered of a serious nature. Very slight rises of temperature or a sensation of tingling in various portions of the body were occasionally noted following the injections, but even these soon disappeared. Possibly, had more patients been injected, other symptoms noted by other observers may have appeared.

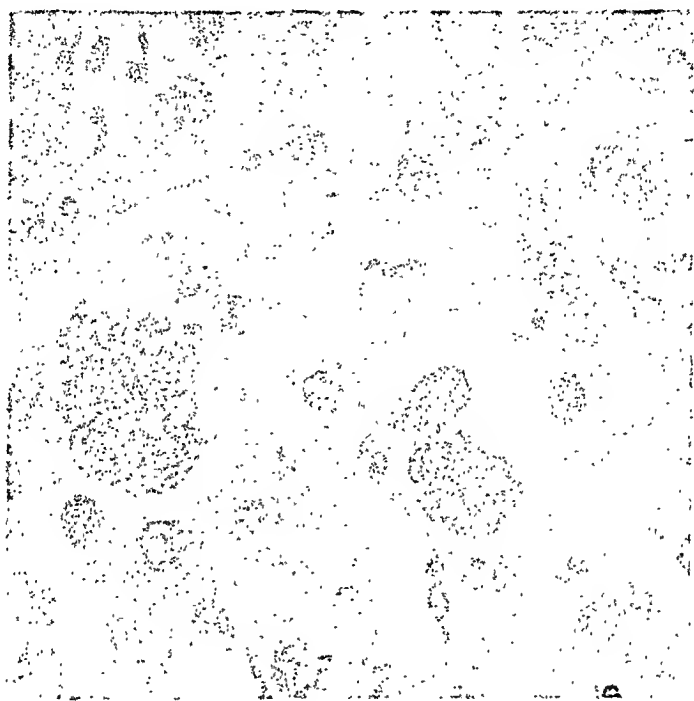


FIG. 8. LIVER OF DOG

Two years and two months following 0.8 cc. thorotrast per kilogram body weight. Aggregation of reticulo-endothelial cells filled with thorium dioxide particles nearly occluding the sinuses. $\times 450$

The tissues of those patients who came to necropsy or operation subsequent to thorotrast injection revealed essentially the same histological pictures as described in the animal tissues, wherein the corresponding dosage was used. Four cases, in particular, presented interesting findings. One was that of a patient with carcinoma of the body of the pancreas and extensive metastases to the liver. The nodules of carcinoma cells, as diagnosed by the

areas of rarification in radiographs ante mortem, were free of any thorium dioxide particles. The surrounding liver substance showed the compressed reticulo-endothelial cells containing a considerable amount of thorium dioxide (fig. 7).

A patient with multiple myeloma with extensive bony metastases, yet no liver involvement, was studied. The myeloma nodules did not show the presence of thorium dioxide in any of

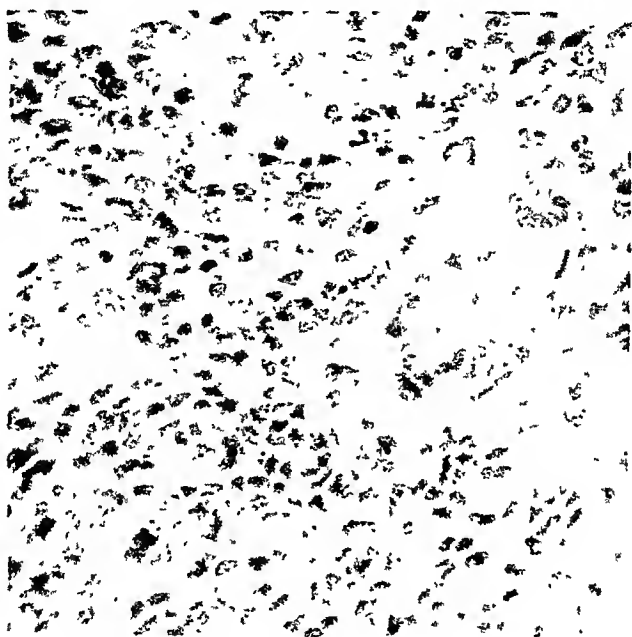


FIG. 9. SPLEEN OF DOG

Two years and two months following 0.8 cc. of thorotrast per kilogram body weight. Reticulo-endothelial cells filled with thorium dioxide particles. Presence of giant cells containing no thorium dioxide particles. $\times 500$

the cells. Radiographs of the liver and spleen, ante mortem, revealed the liver to be homogenously smooth, no areas of rarification being noted.

A patient with multiple hydatid cysts of the liver, seen as well defined areas of rarification in the radiographs, was operated on and the cyst contents and wall mechanically removed. No thorium particles could be demonstrated in the clear cyst contents or the wall. This patient was markedly jaundiced at the time of

injection. However, after the cyst was drained and pressure removed, his jaundice disappeared. The draining sinus of the operative site has not completely healed as yet (fig. 11).

A fourth patient having an amebic abscess of the liver was operated on and the abscess drained, subsequent to thorotrast injection. The contents of the abscess and scrapings of the wall were examined. Vegetative forms of *Endamoeba histolytica* were demonstrated, but no thorium particles were found (fig. 10).

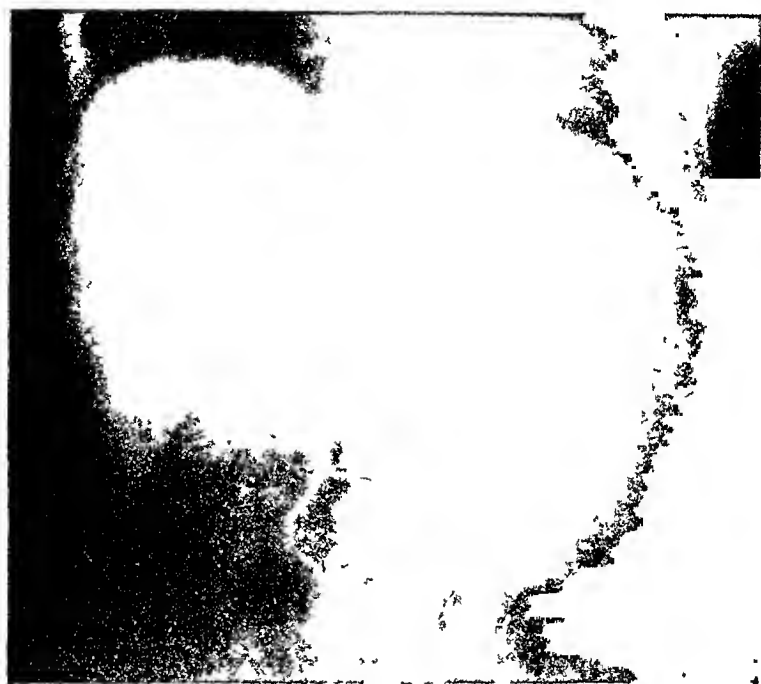


FIG. 10. RADIOGRAPH OF LIVER AND SPLEEN
Case of pernicious anaemia. Marked splenomegaly present

In the tissues studied at different times the thorium particles varied in size. The granules appeared larger in the cells of the tissues taken later after injection; possibly due to aggregation of the particles in the cells.

Identification of the thorium particles by specific chemical stains has not been possible so far. The granules appear as shiny, opaque, minute particles, simulating dark-colored, finely granular, bronze or the pigment of the malarial parasite in both unstained and stained preparations. At times, it is extremely

difficult to differentiate thorium dioxide particles from hemosiderin and other products of hemoglobin metabolism, which are especially present in the spleen. A very useful method is the Berlin blue, or even better, Turnbull's blue tissue staining technique. The thorium particles remain unstained, but the hemosiderin is stained a beautiful clear blue. In my attempts to visualize lymph nodes by the subcutaneous injection of thorium

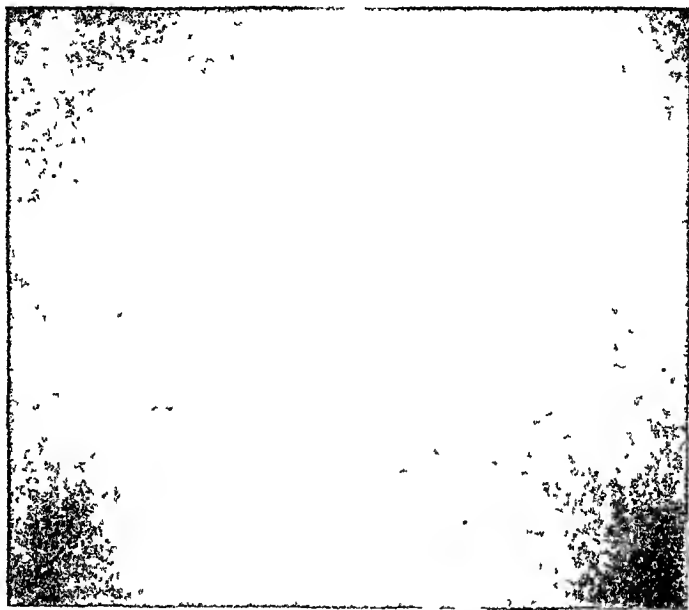


FIG. 11. RADIOGRAPH OF LIVER AND SPLEEN

Case of hydatid cyst of the liver oval area of rarefaction near lower right border clearly visible.

dioxide the draining normal nodes can be discerned rather clearly. However, those nodes in which the metastasizing malignant cells have completely occluded the draining sinuses, no thorium particles can enter, consequently the nodes do not increase their opacity to the roentgen rays. Microscopic examination of some of the nodes containing large malignant metastatic nodules, which were not visualized even after one year, revealed finely granular pigment simulating thorium dioxide. However, staining by the above methods revealed the fact that the particles were not thorium dioxide, but hemosiderin.

DISCUSSION

Upon analysis of this work and that of other investigators, it is strikingly notable, except in certain instances, that the most marked histopathologic changes are noted in the tissues of those animals which were given dosages larger than 0.8 cc. per kilogram of body weight. In other instances, the entire dose was given in one or only two injections, instead of in three or four fractions.

Our results as well as those of other investigators^{2, 29} differ from those obtained by Shih and Jung. In the report of these latter investigators the manner of sacrificing the rabbits was not given nor was any note made concerning control rabbits killed by the same method. Possibly these notes may explain the difference in results. In our work, we did not note any regular instances of marked hemorrhagic tendencies or extravasations of blood into the internal organs. Dosages up to 8.0 cc. per kilogram of body weight divided into three doses, each given one day apart, were not accompanied by death in any instance, except when unsuccessful attempts at intracardiac (intraventricular) injection in the smaller animals resulted in extravasation of the solution into the heart muscle or mediastinum in one or two instances.

Our work, using the different doses, agrees in general with the recent results obtained by Whitaker, Davie and Murgatroyd. The instances of slight differences in results may be explained by the slight difference in dosages in the various animals.

Shute and Davis were not able to visualize the placenta of pregnant animals as reported by previous investigators. Certainly in our own sections, too few reticulo-endothelial cells are present in the placental tissues to fix sufficient thorium dioxide particles which will render the organ visible by the roentgen ray when doses of 0.8 cc. per kilogram of body weight are given. It appears from the work of these authors that doses up to ten times this amount are not sufficient to visualize the placenta roentgenographically. Coincidentally, as seen in the tissues of our experimental animals, intense degeneration of the liver and spleen parenchyma occurs when such large amounts of the substance is used. However, we did not note a single instance of rupture of

the spleen in any of our animals. Indeed, the mechanism of splenic rupture being caused by thorotrast injection would be difficult to explain in the light of present knowledge concerning the anatomy and physiology of the spleen. In the study of the spleen as a reservoir for red blood cells,⁴ it was possible to watch the spleen under the fluoroscope in its dilated phase while the various animals were asleep under the influence of chloralose. One minute after the intravenous administration of adrenalin, contraction of the spleen could be seen very well; the degree of contraction being in accord with simultaneous hematocrit determinations.

The phase of the problem dealing with the radioactivity of thorium dioxide has been of considerable interest. This substance is the salt of the heavy metal thorium, which is classified among the radioactive metals. The Radium Institute of the Academy of Freiburg¹¹ found that 100 cc. of umbrathor which has the same thorium dioxide content as thorotrast contains a quantity of radioactive substance, the gamma-ray equivalent of which is that of the gamma-rays of 1.24×10^{-6} gr. of radium. Thus, since approximately only 50 to 60 cc. is used in the average patient, the entire liver and spleen, principally, are subjected to a total gamma-ray irradiation of 0.62×10^{-6} gr. of radium. As small as this may appear, Stewart and his associates reported that in one case, the spleen after autopsy, when placed on a photographic plate for one day, contained enough radioactive substance to register an image on the plate, while control spleens were negative. These results could not be confirmed by Yater and Otell²⁹ and Baumann and Schilling,² or my own work.

The results reported in this paper indicate that there is a distinct difference, both clinically and histopathologically, when different doses of thorotrast are used and that also the amount of clinical and histological reactions differ in instances wherein the substance is given in one large single dose or divided into three or four fractions, each given twenty-four hours apart.

Those animals whose tissues were examined two years after administration of thorotrast have revealed most interesting results. It is noted that the thorium dioxide granules are collected

almost entirely in the reticulo-endothelial cells which have aggregated in more or less focal areas, almost occluding the capillary sinuses. In the spleen, particularly, the presence of giant cells, not of the foreign body type, diffusely distributed throughout the organ is of distinct histopathologic interest. The results thus far indicate that the presence of the thorium dioxide particles are more or less permanent and the histopathologic changes although slight, are nevertheless present. The findings in the light of other histological pictures which are described can hardly lead one to believe that the method is "absolutely harmless" and can be used indiscriminately in all cases. These results are not in accord with the work of Radt.¹⁹ This author reported that he had been unable to determine any histopathologic changes in the tissues of his animals even after one or two years following administration of thorium dioxide.

ADVANTAGES AND DISADVANTAGES OF THE METHOD

The advantages of the method may be summarized as follows:

(1) Distinct roentgenographic visualization of the liver and spleen is possible, using dosages of 0.8 cc. of thorotrast per kilogram of body weight intravenously. This has been a definite aid in differential diagnosis of abdominal masses or tumors, that is whether intrahepatic, intrasplenic or extrahepatic or extrasplenic.

(2) Metastatic or primary nodules in the liver or spleen when sufficiently large, may be discernible in the radiographs.

(3) Definite evidence of existing hydatid cysts, abscesses, cirrhosis and primary tumors in the liver and spleen can often be gained which evidence is hardly obtainable by any other method.

(4) Splenic motility, that is, contraction and dilatation as affected by various substances, can be studied in a more direct manner.

The disadvantages may be summarized as follows:

(1) A foreign substance is injected intravenously which is "fixed" by the reticulo-endothelial cells.

(2) Initial clinical reactions have been noted although these are neither consistent nor severe.

(3) Concomitant histopathologic changes, slight as they may appear, have definitely resulted in the reticulo-endothelial cells.

(4) The tissue studies, after two years, seem to indicate that the thorium particles remain more or less permanently in the reticulo-endothelial cells.

(5) Even should the radioactivity of the thorium dioxide particles be proved to be negligible, the mere presence of them as foreign bodies for so long a time, should not permit of the routine use of the method in cases other than the type noted.

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A NOTE ON ACETONE INSOLUBLE LIPOIDS IN RELATION TO ANTIGEN FOR THE WASSERMANN REACTION

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While the exact chemistry of alcoholic extracts of beef heart or other tissue in relation to antigen for the complement fixation and precipitation reactions in syphilis is as yet unknown, it is generally agreed that those lipoids soluble in alcohol and ether but insoluble in acetone possess a high degree of antigenic sensitiveness. These are believed to be mainly tissue lecithins or diaminomonophosphatids and are commonly referred to as "acetone insoluble lipoids." Curiously however, such lipoids extracted from egg-yolk or other substances do not possess the same sensitiveness as those derived from tissues so that the latter continue to be employed in the preparation of antigens for both the Wassermann and various precipitation procedures used in the serum diagnosis of syphilis.

In view of the remarkably high antigenic sensitiveness of these tissue acetone-insoluble lipoids it has occurred to us worth while to inquire into the possibilities of increasing the sensitiveness of antigen for the Wassermann reaction by endeavoring to prepare extracts carrying more than the usual amounts while keeping the cholesterol content at approximately 0.2 per cent in order not to increase the danger of falsely positive and non specific reactions attending the use of larger amounts of this sensitizing agent.*

When 25 grams of desiccated beef heart powder (Digestive Ferments Company) are extracted with 200 cc. of ether at room temperature for five days followed by extraction with 200 cc. of ethyl alcohol at 37°C., for four days, about 2.2 grams of acetone

* Unpublished data.

insoluble lipoids are recovered by precipitation of the ether and alcoholic extracts with an excess of acetone. When all of these along with 0.2 gram of cholesterol are dissolved in ether and added to a secondary alcoholic extract (100 cc.) of the tissue, the antigenic unit by the Kolmer method of titration is approximately 0.5 cc. of 1:2400. When 4.4 grams or double the amount of lipoids are added to 100 cc. of the secondary alcoholic extract along with 0.2 gram of cholesterol, the antigenic unit is raised to about 0.5 cc. of 1:2800 to 1:3000 with no increase of anticomplementary activity which is generally about 0.5 cc. of 1:4 for both antigens. However not all of the acetone insoluble lipoids in the second extract go into complete solution nor can solution be obtained by immersing the antigen in a water-bath at 56° to 75°C. as employed for aiding the solution of large amounts of cholesterol. But results have indicated that it is possible to increase the specific antigenic sensitiveness of extracts by increasing the amounts of acetone-insoluble lipoids.

When 2.2 grams of Pfanstiehl's ovo-lecithin was added to 100 cc. of a secondary alcoholic extract along with 0.2 per cent cholesterol, the antigenic unit was only 0.5 cc. of 1:800 while double this amount (4.4 grams) to 100 cc. of extract along with the same amount of cholesterol gave an antigenic unit of 0.5 cc. of 1:1200. It is apparent therefore that ovo-lecithin is not as antigenic as tissue lipoids; furthermore these extracts were sometimes slightly hemolytic in a dose of 0.5 cc. of 1:4 but showed no increase of anticomplementary activity.

In Noguchi's later method of preparing acetone-insoluble lipoids, beef heart is first extracted with acetone to remove hemolytic and anticomplementary substances and the dried residue then extracted with absolute ethyl alcohol to secure the alcohol soluble lipoids. In one experiment we extracted 30 grams of desiccated beef heart (Digestive Ferments Company) with 100 cc. of acetone for five days at room temperature. The acetone was discarded, the tissue dried and extracted with 100 cc. of absolute ethyl alcohol for five days at room temperature. A portion of this extract was sensitized with 0.2 per cent cholesterol

and was found to give an antigenic unit of 0.5 cc. of 1:6000 by the Kolmer method of titration. A second portion of 50 cc. was evaporated by fanning to 25 cc. to concentrate the lipoids and sensitized with 0.2 per cent cholesterol. This extract had a slight sediment of undissolved lipoids but gave an antigenic unit of 0.5 cc. of 1:8000 which appeared to be due to the higher concentration of alcohol soluble lipoids since the amount of cholesterol was the same in both. Neither extract was hemolytic in dose of 0.5 cc. of 1:4 and the anticomplementary unit of each was 0.5 cc. of 1:4 so that an increase of antigenic sensitiveness was secured without an increase of non specific complement fixation.

Somewhat similar results were obtained by extracting 25 grams of the desiccated beef heart with 100 cc. of absolute acetone free ethyl alcohol for five days at room temperature without the preceding extraction with acetone. When a portion of this extract was sensitized with 0.2 per cent cholesterol, the antigenic unit was found to be 0.5 cc. of 1:6000. When 40 cc. was concentrated to 20 cc. by fanning and sensitized with 0.2 per cent cholesterol there remained a slight residue of undissolved lipoids but the antigenic unit was increased to 0.5 cc. of 1:8000. Both extracts were entirely free of hemolytic and anticomplementary activity in amounts as high as 0.5 cc. of 1:4.

It would appear possible therefore to increase the sensitiveness of antigens for the Wassermann test by increasing the amounts of lipoids contained in alcoholic tissue extracts without increasing the amount of cholesterol beyond 0.2 per cent which is inadvisable at least in those methods employing a primary incubation of fifteen hours or longer at 6°C.* Furthermore we have observed that such antigens, as briefly described herein, may be used in a dose of 20 antigenic units in the Kolmer modification of the Wassermann test with increased antigenic sensitiveness and with no falsely positive or non specific reactions with non syphilitic sera since such a dose is still from forty to eighty times less than the anticomplementary amounts and representing therefore an extremely wide and safe range.

* Unpublished data.

CONCLUSIONS

(1) It is possible to increase the specific antigenic sensitiveness of alcoholic extracts of beef heart by increasing the amounts of alcohol soluble but acetone insoluble lipoids.

(2) Such extracts sensitized with no more than 0.2 per cent cholesterol possess a very high degree of antigenic sensitiveness with no increase of non specific or anticomplementary properties.

(3) Antigens of this kind permit the use of larger amounts in conducting the Wassermann test with an increase of specific sensitiveness for syphilis antibody.

EDITORIAL

"YAWS AND SYPHILIS. TWO DISEASES OR ONE?"

Under the above caption, D. B. Blacklock, M.D., Professor of Parasitology, School of Tropical Medicine, University of Liverpool, gives such a well-reasoned and convincing analysis of this vexed question that the Editor of Tropical Diseases Bulletin, in the November, 1933 issue in which his paper is published, is moved to remark in a foot-note as follows: "Professor Blacklock questions the soundness of much of the current beliefs about yaws and its relation to syphilis. His arguments will doubtless receive open-minded attention."

In a paper published by the undersigned in collaboration with Lieut. Comdr. Edwin Peterson (MC) U. S. Navy some five years ago, one of the conclusions arrived at was that medicine was already in possession of sufficient facts to settle this matter in favor of unity (Professor Blacklock uses the term "unicity," a word in use when gonorrhoea was also thought to be a part of syphilis), if only we would apply a little logical reasoning to these known facts. Professor Blacklock has applied this logic with meticulous precision.

In his estimable work, "Diagnostics and Treatment of Tropical Diseases," Fifth Edition published by P. Blakiston's Son & Co. in 1929, Admiral Stitt used the same technique as Professor Blacklock, that is, he took the table of points purporting to differentiate syphilis and yaws and defeated "duality" in detail. In addition, Stitt published pictures from known yaws cases, showing aneurysms of the aorta and detailing findings in framboesia which completely stultified the differential tables. Tropical Diseases Bulletin in its review of Stitt's book at the time of the appearance of this Fifth Edition, after observing that it would find its way on to the shelves of all American (sic!)

practitioners in the tropics, used the following words in describing his treatment of yaws:

The author, influenced by workers in Haiti, has elected to accept the evidence in favour of the identity of yaws and syphilis, which in the present state of our knowledge seems unwise. He then describes yaws shorn of some of its well known characteristics but clothed in rather unusual garb decked out with visceral lesions, hepatic gummata, aneurysms and cerebral haemorrhage. The text bears evidence of rather hasty compilation and critical analysis reveals numbers of minor omissions and little inaccuracies and some looseness of expression. After such a statement it is only fair to give examples of these blemishes which will no doubt disappear in subsequent editions. Secondary yaws lesions on the trunk are said to be rare, which is not true.

After this rather rollicking review, it will be a source of satisfaction to physicians everywhere that Stitt's views are beginning to find justification even in quarters formerly hostile to them.

It is to be regretted that the Profession in Great Britain had to permit a logician to show them the folly of their course in fighting over this question. It has already been pointed out* that

Yaws then means a certain definition, fallacious withal, into which must be crowded all those cases of syphilis which appear to omit certain well-recognized symptoms of the European disease.

From the days in the seventeenth century when Thomas Sydenham sized up this matter correctly down to the present time, every generation has seen one or more English physician whose research and reasoning were correct upon the venereal diseases. The names of Benjamin Bell, Berkeley Hill, and Jonathan Hutchinson, to mention only three, will forever be remembered when the venereal diseases are under discussion. The last named was not only one of the greatest syphilographers of all time but his brand of logic when trained upon the yaws-syphilis question left nothing to be desired. It should not have taken all this time for truth to prevail. The acceptance of truth is, however, a slow process oftentimes. The rancor engendered by the hero-worshippers requires time to die down. Professor Blacklock quotes many

* BUTLER, C. S.: Diagnosis and treatment of Yaws. *Internat. Clinics*, ser. 40, 2: 1-14. 1930.

more or less important writers along the line of the yaws-syphilis investigations but has little to say about his own countrymen who have born the brunt of it in defence of what they knew to be true. Nor aught but silence has he for the group of Americans, principally U. S. Naval Medical Officers, who have, by research and writing, defended Hutchinson's views for the past thirty years.

The challenge issued in the *Lancet* of April 25, 1931 is still in order. Here it is: "Yaws is purely an artefact over the disease syphilis. This statement is made with the hope that some dualist will produce evidence to the contrary." There has been ample time for all such evidence to assert itself.

—C. S. BUTLER.

NEWS AND NOTICES

FURTHER EXPERIENCE WITH THE FRIEDMAN HORMONE TEST FOR PREGNANCY

The Committee on Research is pleased to make the following report concerning further experience by members of the American Society of Clinical Pathology with the Friedman hormone pregnancy test.

From the reports, there is evidently a tendency for the rabbits to be observed after a forty-eight hour period instead of a twenty-four hour period as first proposed for the test. Some men inject the rabbits more than once and many rabbits are observed at operation and used again later. Table 1 is a compilation of results reported during 1933 and there is combined with these reports those previously reported. This makes a grand total of 5,759 cases in which there were 152 erroneous results or 2.63 per cent. There is a 1 per cent error in reporting positives and 4.6 per cent error in reporting negatives; 0.3 per cent were reported as doubtful. This indicates that the test is accurate in 97 per cent of the trials. The test shows up well in chorioepitheliomas and hydatid moles where twenty-five out of twenty-six cases were positive. The instance of positive tests in ectopic pregnancy is 77.6 per cent for the combined groups. About 1.5 per cent of the animals die before the test is completed.

Table 2 indicates the tests which have been run in an attempt to diagnose tumors of the testis. Ferguson, in a recent paper, has pointed out the inadvisability of using the rabbit in testing for this condition and demonstrated the necessity of a quantitative test. Nevertheless, it is interesting to note that in eight instances out of nineteen trials, the test was positive.

Many members of the American Society of Clinical Pathology have received a complimentary copy of "Dehydrated Culture Media and Reagents" issued by the Difco Laboratory of Detroit,

TABLE 1
FRIEDMAN HORMONE PREGNANCY TESTS

TOTAL TESTS	POSITIVE	NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE	ECTOPIC-POSITIVE	ECTOPIC-NEGATIVE	CHORIOEPITHELIOMA OR HYDATID MOLE- POSITIVE	CHORIOEPITHELIOMA OR HYDATID MOLE- NEGATIVE	EARLIEST TEST, DAYS AFTER LAST MENSTRUATION	AMOUNT OF URINE (EACH INJECTION)	NUMBER OF INEC- TIONS	DURATION OF TEST hours	ANIMALS DYING FROM INJECTIONS	DOUBTFUL	CLINICAL PATHOLOGIST REPORTING
13	6	6					1	*	11	cc.	3-4	36-48	3	1	Dr. R. C. Beck, Richmond, Virginia
160	80	77		1	1				33	8-10				3	Dr. A. G. Foord, Pasadena Hospital, Pasadena, California
9	4	5							10	10	2	96			Dr. H. A. Heise, Uniontown, Pennsylvania
86	77	9							10						Dr. G. B. Kramer, Youngstown Hospital, Youngstown, Ohio
109	61	48			4		1		33	12 ¹	1	36-48	1		Dr. Seab J. Lewis, Beaumont, Texas
67	31	36	1	5	2				29	10	3 ²	48	3		Dr. O. W. Lohr, Saginaw, Michigan
109	42	67		2					7						Dr. J. M. Moore, San Antonio, Texas
16	11	5							21						Dr. H. R. Prentice, Bronson Hospital, Kalamazoo, Michigan
289	129	160	5	4	4	4	4		8	10	2 ³	48	8		Dr. W. M. Simpson, Miami Valley Hospi- tal, Dayton, Ohio
37	16	21							10	10	1	48			Dr. M. Warren, Maine General Hospital, Portland, Maine
260	134	121	2 ¹	2	2		1		31	10	2	48	7	5	Dr. A. M. Young, Mount Sinai Hospital, Cleveland, Ohio
1,164	600	555	8	14	13	4	7						22	9	
4,595	2,526	2,058	24	106	88	25	18	1					72	11	Previously reported—Am. Jour. Clin. Path., 3: 97-102, 1933
5,759	3,126	2,613	32	120	101	29	25	1					94	20	Grand total

* Two tests negative in patients with hydatid mole after operation. Two tests negative in patients with chorioepithelioma after operation.

¹ Catheterized specimens.

² Twelve hours apart.

³ Six hours apart.

⁴ One case teratoma ovary, 1 case tuberculosis of genital tract.

Michigan. This is the fourth edition of this extremely useful manual and copies of it may be procured by addressing the Director of the Laboratory.

Dr. J. H. Black is Chairman of a committee engaged in revising the Constitution and By-laws of the Society. Any member of the

TABLE 2
FRIEDMAN TESTS IN TUMORS OF THE TESTIS

TESTS DONE	POSITIVE	NEGATIVE	REMARKS	CLINICAL PATHOLOGISTS REPORTING
2	1	1	Positive in case of tumor removed 12 days previously, negative in case of seminoma removed 2 years previously.	Heise
2	2		1—teratoma testis; 1—seminoma testis.	Lohr
7	3	4	3 positive in cases of embryonal carcinoma of testis—(microscopic diagnosis), 4 negatives in suspected malignancy testes.	Simpson
2	2		2 tests on same patient with teratoma testis.	Young
4		4	3 cases of seminoma, 1 case of teratoma testis.	Foord
1		1	2 months after operation for teratoma testis.	Lewis
1		1	2 days after operation for teratoma testis.	Moore
<hr/> 19	<hr/> 8	<hr/> 11		

Society who has any suggestions to make concerning the Constitution and By-laws is urged to get in immediate communication with Dr. Black.

The Thirteenth Annual Convention of the American Society of Clinical Pathologists will be held in Cleveland on June 8, 9, and 10. The Headquarters is the Hotel Cleveland. The manager of the Hotel will send members of the Society returnable postcards for reservations. It is urged that these reservations be made as promptly as possible. The Hotel has set aside 150 rooms for members of the Society which will be held until April 1.

The Program Committee has made up a very fine program for the Convention, a feature of which will be "Cancer Diagnosis." It is hoped that a large attendance will be present.

Members are urged to make early application for space for their scientific exhibits in order that the local committee may make proper arrangements. Members desiring to read papers on the program should forward their titles to the Secretary not later than April first.

Drs. Warren T. Vaughan and Walter Simpson have been appointed to the Editorial Board of the Journal. They replace Drs. Keilty and Lynch whose terms expire.

THE PATHOLOGY OF PSITTACOSIS

DISCUSSION WITH CASE REPORT*

ALVIN G. FOORD

From the Laboratories of the Pasadena Hospital, Pasadena, California

From April 1931 to April 1933 fifty-seven cases of proved psittacosis have occurred in California, forty-seven cases in other states of the union, and during the years 1929 to 1933 approximately eight hundred cases with a mortality rate of 35 to 40 per cent occurred throughout the world (Meyer⁷). Furthermore recent studies, especially those of Meyer and by Hasseltine² have shown that birds of the parrot family commonly harbor the virus of the disease and spread the condition to other birds and possibly to man, without showing any symptoms of the disease themselves. Consequently psittacosis must be considered as a possible menace in localities where parrakeets or parrots are raised or sold, and in the entire country, unless the present regulations against interstate commerce in parrots, parrakeets and love birds are strictly enforced.

In spite of the large numbers of cases in recent years and in the epidemics of the past, beginning with the description of the disease by Ritter of a small group of cases in Uster, Switzerland in 1879, very few post mortem reports have been made. However, even from the first description by Eberth¹ of the pathology in Ritter's cases, it is obvious that there is a distinct post mortem picture, primarily that of a unique pneumonic process in the lungs. Eberth described the lungs as showing a gray-red sero-croupous hepatization, part lobar and part lobular, in which the exudate was a loose meshwork of fibrin with a few cells. Cloudy swelling of the parenchymatous viscera and a few petechiae on

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

the serous surfaces were also found. Leichtenstern's⁴ case, showed a lobar consolidation of both lower lobes and of the dependent portions of the upper lobes. Surfaces made by section showed a soft, smooth, dull, homogeneous appearance, and microscopically a serocellular or cellulofibrinous exudate. In 1930, Siegmund¹³ restudied the sections from the case of Leichtenstern and found changes similar to those he describes in his own case of 1929. In the latter a peculiar soft, edematous pneumonia involved both lobes of the left lung and the right lower lobe. The pleura was smooth and the sectioned surface smooth and dark red in color. There was no bronchial or peribronchial involvement. Microscopically the diseased lung showed all stages from a simple edema to complete filling of the alveoli with cells, almost entirely large mononuclears. Fibrin content was slight to moderate. The bronchi were particularly spared. In addition, a moderate acute splenic tumor (280 grams) and cloudy swelling of the liver and kidneys was seen. Microscopically there were large numbers of endothelial cells in the pulp of the spleen, and in the liver the Kupffer cells were increased in number, loosened from the capillary walls, and in some places formed small nests of cells. There was a marked edema of the leptomeninges with increase of histiocytes in the subarachnoid space. The brain was edematous but otherwise not remarkable. An inflammatory hyperplasia was seen in the lymph nodes of the chest, but elsewhere they were not enlarged.

Similar involvement of the lungs was reported by Wilson,¹⁵ Maclachlan et al.,⁶ Turnbull,¹⁴ Peterson et al.,⁹ and Polayes and Lederer.¹⁰ Wilson found the bronchioles to be practically spared in the process while Maclachlan, Turnbull and Polayes and Lederer found an inflammatory process in their walls, characterized by infiltration with large mononuclear cells, chiefly. Microscopically all the above authors described the consolidation in the lung as being due to an exudate containing varying amounts of fibrin and large mononuclear cells. Polymorphonuclear cells were absent or rare unless secondary infection was present. Of most striking interest were the changes in the alveolar walls in all the recent cases described and stressed by Maclachlan as being

the most distinctive feature of the disease. This consisted of a marked proliferation with enlargement of the cells lining the alveoli, causing the alveolar walls to appear thick. Turnbull's patient showed also hemorrhage in moderate degree into the alveoli and in the most advanced stage thrombosis of capillaries and even of the smaller arteries of the lung. Some alveolar capillaries were thrombosed in Maclachlan's and Polayes' cases.

Oberndorfer's⁸ patient who died thirty days after onset, showed large portions of several lobes involved with a hepatization, but obviously a secondary bronchopneumonia was present. Horder and Gow's² patient, likewise showed findings characteristic of a secondary infection. The same was true of Russell's¹² patient, who in addition showed aspiration of stomach contents into the respiratory apparatus.

Rivers et al.¹¹ by insufflation of virus-containing material into the bronchial tree of monkeys produced a bacteria-free lesion corresponding to the findings in human cases, especially characterized by proliferation of alveolar epithelium and proliferation or infiltration of alveolar walls with mononuclear cells, producing a decrease of the alveolar spaces by the thickened walls. The alveoli contained serum, fibrin, and larger mononuclear cells.

The findings reported in organs other than the lungs are not striking. Cloudy swelling of the kidneys and liver, moderate soft swelling of the spleen with proliferation of endothelial cells in the pulp is the rule. Peterson et al., however, found multiple areas of focal necrosis in the liver, many showing polymorphonuclear leucocytic infiltration in the centers and midzones of the lobules. The gastrointestinal tract and the lymphatic system appeared to be relatively spared. Waxy degeneration and hemorrhage into the rectus abdominus muscle was described by Turnbull. Ring hemorrhages in the brain and cord are mentioned by the same author, and as being present in the cord by Polayes and Lederer. Peterson et al. described large ring hemorrhages of various ages in the caudate nucleus and punctate hemorrhages in the corpus callosum, internal capsule and thalamus and floor of the fourth ventricle. Degeneration of myelin in the immediate neighborhood was present.

Although the disease appears to primarily attack the lung in human cases, the lungs are practically spared in birds and in white mice, the latter of which serves as the best test animal for laboratory diagnosis. In birds the most important finding is a large swollen liver and spleen. In mice likewise both organs are enlarged and in addition multiple minute foci of necrosis in the liver are usually found. Only occasionally are necrosis found in bird's livers. Rickettsia-like intracellular organisms called by Lillie "Rickettsia psittaci" or "L.C.L. bodies" by Meyer after the three investigators, Lillie, Coles and Levinthal, who described them about the same time, are best demonstrated in smears from the necrotic areas in the liver or the exudate from the peritoneum of inoculated mice. Smears and sections from human material less frequently show these "minute Gram negative intracellular coccoid and bipolar bacilliform bodies of about 1.2 to 0.3 micra diameter, found in reticulo-endothelial cells, mesothelial cells and large mononuclear cells of the parrot, and in large mononuclear cells in man" (Lillie⁶).

Within the last year two human cases were examined at necropsy by the author. These were the only two who died in a series of ten cases studied clinically by Dr. James B. Luckie, to whom I am indebted for the clinical history. One patient developed an acute exacerbation of an old pyelocystitis and died primarily from this. In addition, there was bilateral secondary confluent broncho-pneumonia which complicated the lung picture so that only one small focus of the original psittacosis remained. The pathology of this was similar to that described in the case below, and further description will not be made. The other patient died of his psittacosis primarily. Material from the lung in both cases, and the liver and spleen in the case below produced typical lesions in mice, the inoculation experiments being made by Dr. Karl Meyer of the Hooper Foundation, San Francisco.

CASE REPORT

Mr. T. J. T., age 57, two weeks before the onset of his illness had purchased two dozen love birds and built an aviary in his yard in order to raise them for commercial purposes. Six of the birds, after developing a diarrhea and a cough,

died shortly after their purchase. On February 10, 1932, the patient abruptly developed a fever, severe headache, and a slight cough. The next day the cough, was more severe, but he could not raise any sputum, nor did he raise sputum at any time until the last three days of his illness. His chest felt "as if it had a tight band around it." His fever and cough persisted, the headache became more severe, and on the fifth night of his illness he became delirious, got out of

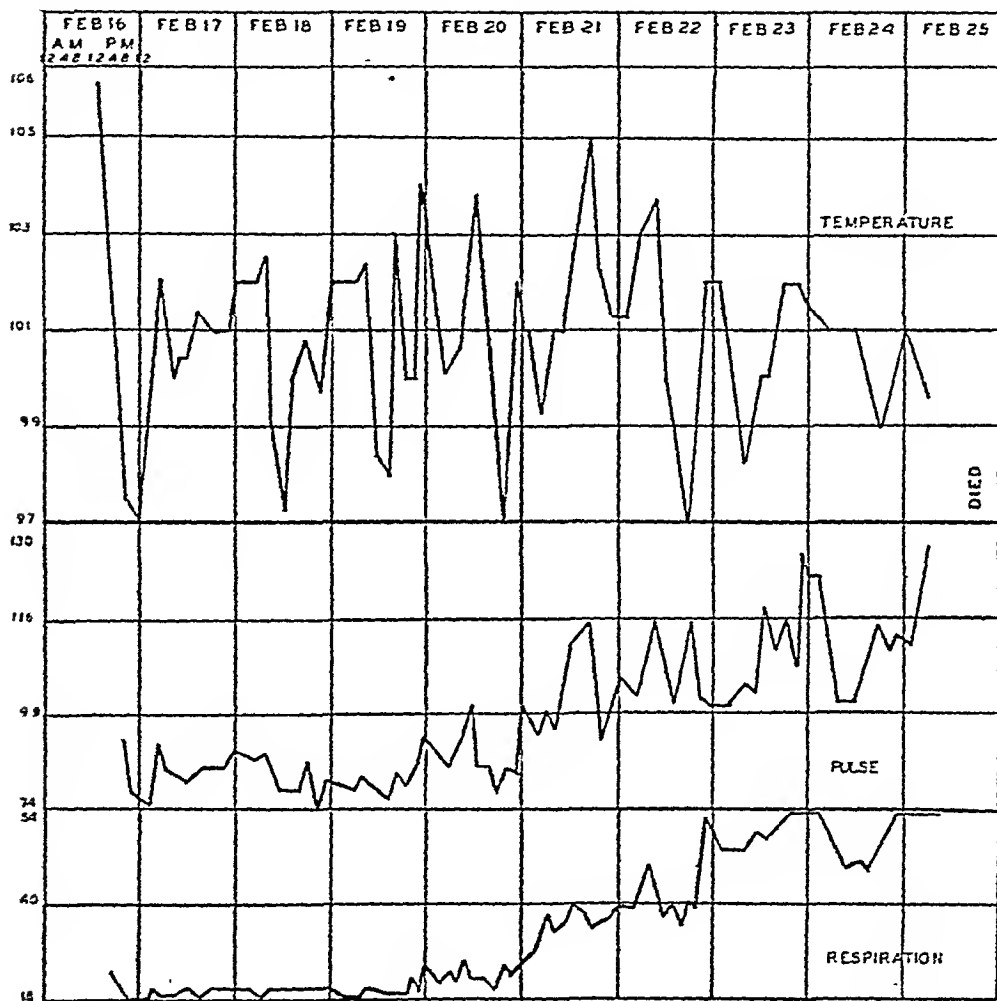


CHART 1. TEMPERATURE, PULSE AND RESPIRATION CURVES OF CASE OF PSITTACOSIS

bed and tried to find his way out of the house. His wife, who became ill two days after her husband, finally restrained him and led him back to bed where both fell exhausted. He was not seen by a physician until two days later, when he was examined by Dr. Luckie. At that time he was semi-delirious, his pulse 100, temperature, rectal 106°, respirations 24. He appeared exhausted, his eyes blood-shot. There was slight redness of his throat. The lymphatic nodes in

his neck were not enlarged. Examination of the chest showed nothing abnormal anteriorly. Posteriorly there was no dullness on percussion, but on auscultation the breath sounds were greatly diminished on the left base, extending upward to the angle of the scapula. Fine dry râles were heard in this area. A few spots resembling rose spots were found on the abdomen.

During the next few days the patient became more delirious, the harrassing cough and fever persisted, dyspnea developed, and râles were found over the entire chest. Death occurred on February 25, fifteen days after the onset of the illness. Chart 1 shows the temperature, pulse and respiration. It will be noted that only in the last few days of the illness was there any marked increase of pulse and respiration, in spite of the high temperature. Necropsy was performed two hours after death.

NECROPSY REPORT SUMMARY

The only external feature of note is a moderate posterior lividity and cyanosis of the nailbed and lips. The head, neck and spinal canal were not dissected because of restrictions. When viewed from below the organs of the neck show nothing of interest. The principal findings are in the chest. Each pleural cavity contains about 500 cc. of clear straw colored fluid, and the pleural surfaces are everywhere smooth and glistening. The left upper lobe is large and is extremely heavy. The anterior rim of its lower half contains air and crepitates normally, but the remaining dependent portion occupying over two-thirds of the lobe is uniformly consolidated, moderately firm to the feel, but yields under pressure without tearing. Surfaces made by section (fig. 1) show a uniformly smooth, almost glassy consolidated parenchyma, gray in color with a faint shade of blue. A considerable amount of fluid with very little blood exudes on pressure. There are no granular portions or areas of hemorrhage. The left lower lobe is only about half the size of the upper, is moderately compressed by the fluid in the chest, and on section shows no consolidation—although considerable blood and some fluid exudes from the boggy sectioned surface.

The right upper lobe, except for the anterior rim and lower border, which crepitates well, resembles the left upper. The mid lobe is unchanged, but the upper half of the right lower lobe posterior is uniformly consolidated, and surfaces made by section are airless, glassy, waterlogged, and gray to gray-pink in color.

The tracheobronchial tree shows a reddened mucosa and the larger branches especially contain a large amount of chunky gray exudate, chiefly fibrin, without mucus or pus. There is a slight soft swelling of the tracheobronchial and hilar lymph nodes.

The pericardial sac contains 100 cc. of clear straw colored fluid and the surfaces are smooth and glistening. The only noteworthy changes in the heart are a diffuse dilatation of all the chambers and a dull, opaque, flabby musculature. Moderate coronary sclerosis is present in the larger trunks.

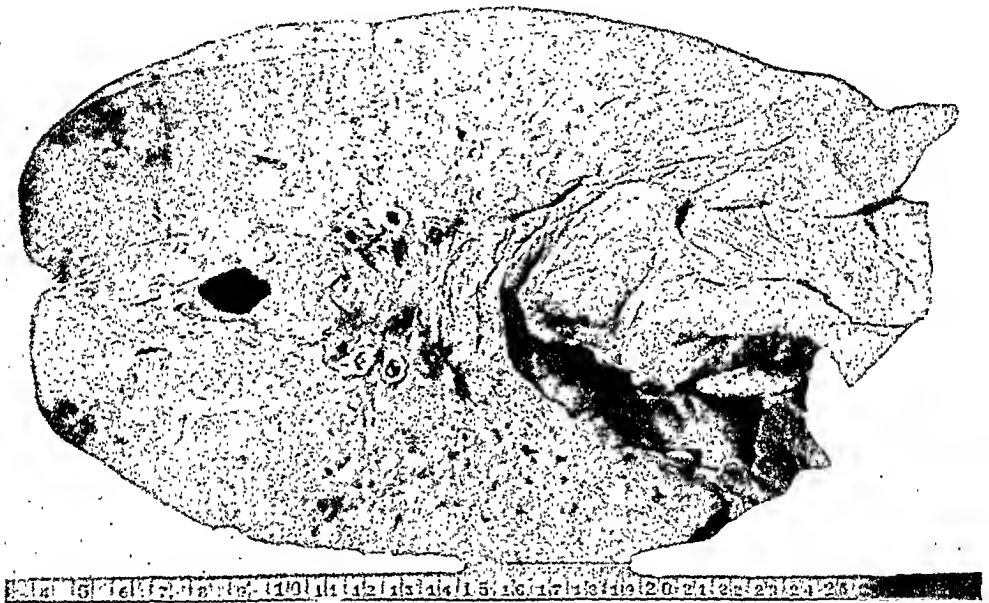


FIG. 1. LEFT UPPER LOBE OF LUNG. SECTIONED FROM APEX TO BASE, SHOWING UNIFORM CONSOLIDATION

There is no fluid in the abdomen. The entire gastrointestinal tract shows a moderate cyanosis of the mucosa. The liver weighs 1900 grams, its edges are rounded, it is firm, purple-chocolate in color, and on section exudes much blood. The lobular markings are plain and the parenchyma is tense and not easily crushed. The gallbladder, ducts, pancreas and adrenals show cyanosis only.

The kidneys are moderately swollen and weigh 325 grams. They show nothing of interest except a diffuse cyanosis. The

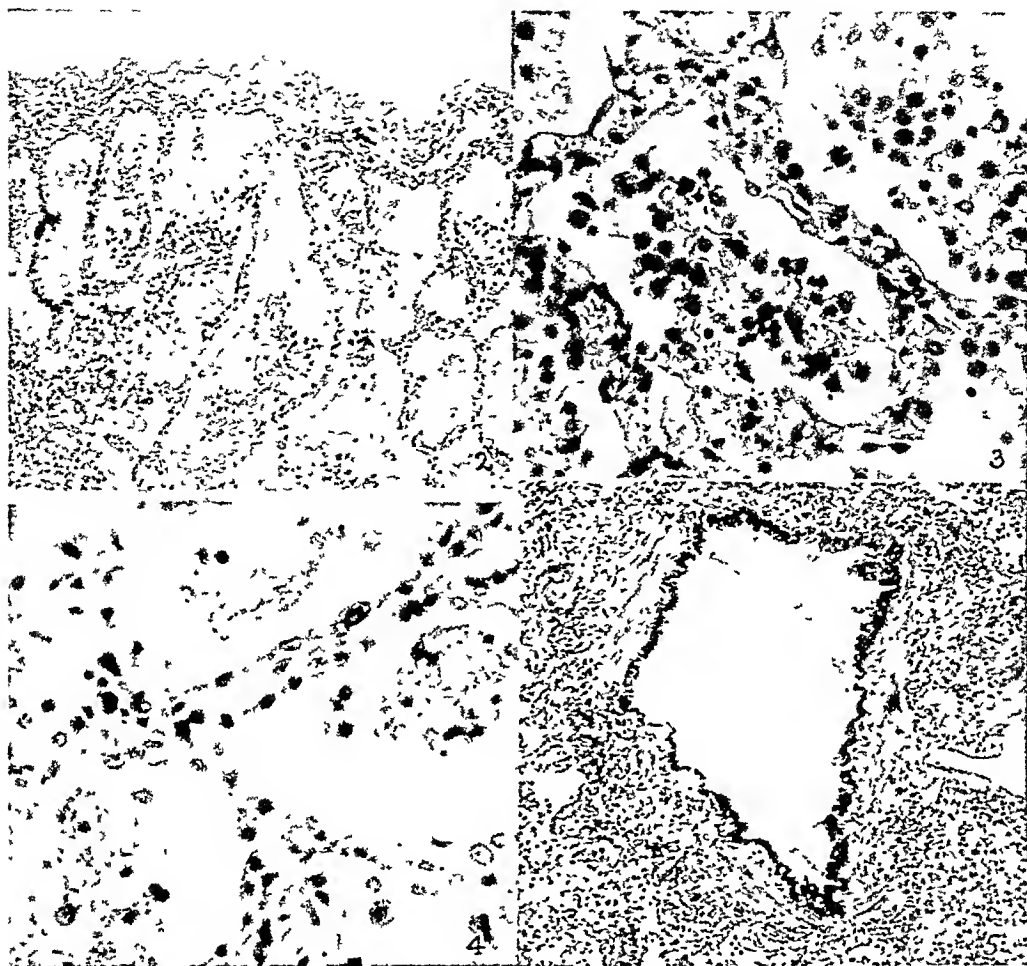
pelves, ureters and bladder likewise show moderate cyanosis of the mucosa. The prostate shows a moderate adenomatous hypertrophy. The testicles and vesicles are free from change.

The spleen is only slightly enlarged and weighs 230 grams. It is somewhat softer than normal, the capsule tense, cuts easily, drains considerable blood, and shows a red-gray, soft pulp from which chiefly blood can be seraped. The Malpighian bodies are just visible.

Moderate arteriosclerosis is present in the aorta and its major branches. The vena cava, iliac and femoral veins are free from thrombi, but fresh thrombi fill the plexus of veins on the left side of the prostate.

HISTOLOGY

Sections through the uniformly consolidated portions of the mid part of the right and left upper lobes show practically similar changes. The alveolar spaces are moderately decreased in size and all are filled with an exudate composed largely of serum with varying amounts of fibrin, which forms a coarse or fine network throughout the entire lumina of the air spaces (fig. 2). Enmeshed in this fibrin are varying numbers of cells, for the most part only moderate numbers, although some alveoli are fairly packed with cells. In all the alveoli the predominating cell is a large mononuclear cell with a single ovoid or bean-shaped nucleus and abundant cytoplasm (fig. 3). Many show phagocytosis of nuclear material, some of red cells, and a few show dust particles. They appear to be typical macrophages. Only rarely is a polymorphonuclear leukocyte seen, although occasional lymphocyte and plasma cell is seen. Erythrocytes are found in small numbers only in a small proportion of the alveoli. Of striking interest is the thickening of the alveolar walls, which appear to be stiff. This is partly due to a congestion in the capillaries, but also largely due to proliferation of histiocytes and to a swelling and prominence of the alveolar epithelium (fig. 4), which shows in many places desquamation of cells into the lumina of the alveoli. The pleura is free from change except for some slight proliferation of histiocytes in its deepest



FIGS. 2 TO 5

FIG. 2. LUNG, INCLUDING PLEURA. SHOWING PLEURA FREE FROM INFLAMMATION. UNDERLYING ALVEOLI FILLED WITH SEROFIBRINOUS, CELL (—) POOR EXUDATE. (LOW POWER)

FIG. 3. LUNG. SHOWING FIBRIN AND LARGE MONONUCLEAR CELLS IN ALVEOLAR EXUDATE. ALVEOLAR WALLS THICK. PROLIFERATION OF ALVEOLAR EPITHELIUM PRESENT. (HIGH POWER)

FIG. 4. LUNG. SHOWING PROLIFERATION OF ALVEOLAR EPITHELIUM AND EXUDATE IN LUMEN. (HIGH POWER)

FIG. 5. LUNG. SHOWING SMALL BRONCHIOLE WITH MINIMAL INFLAMMATORY INVOLVEMENT OF WALL, SURROUNDED BY INVOLVED ALVEOLI. (LOW POWER)

layer, adjoining the involved alveoli (fig. 2). There is no noteworthy hyperemia or cellular infiltration or fibrin deposition. The bronchi in these portions show relatively little involvement (fig. 5). The epithelium is intact in most, and there is an exudate in the lumina composed largely of fibrin and mononuclear cells with a small number of polymorphonuclear leukocytes. There is a slight infiltration of the epithelium with the latter cells in some, and others show a slight infiltration of the subepithelial tissues with polymorphonuclear leukocytes and large mononuclear cells, but some bronchioles are practically entirely spared from inflammatory changes.

Sections taken through the most dependent portions along the lower rims of both the right upper and the right lower lobes show some alveoli which are practically free from exudate, but most of the alveoli contain an exudate which contains only a small amount of fibrin, forming a loose meshwork in which large mononuclear cells are present in moderate numbers, but in addition there are fully as many polymorphonuclear leukocytes. Similar exudate is present in the small bronchi, but here again infiltration of the bronchial walls is not a prominent feature, and many show intact epithelium. The left lower lobe shows the alveoli free from exudate. The alveolar walls are somewhat thickened due primarily to a hyperemia in the capillaries. The alveolar epithelium is fairly prominent in some of the alveoli, and histiocytes are found in the walls in increased numbers, and occasionally about small blood vessels there are fairly numerous histiocytes and young fibroblasts. In a few alveoli, there is evidence of recent organization of the exudate. The bronchi in this portion are not involved. A fairly recent thrombus is found in one of the medium sized pulmonary arterioles and in a few smaller arterioles.

The spleen shows a distinct swelling of the pulp, due partly to congestion and also partly to an increase in the cells, which are chiefly endothelial cells. Many of these are seen in the sinuses and definite endothelial proliferation is found in the walls of the sinuses. Phagocytosis of red cells is seen fairly commonly. A moderate number of plasma cells, lymphocytes, and a few poly-

morphonuclear leukocytes are also found. There is marked encroachment of the pulp on the Malpighian bodies. The arteriolar walls show a moderate hyaline thickening.

The liver shows only a moderate prominence of the Kupffer cells, some of which are swollen and show evidence of phagocytosis of erythrocytes. There is a moderate albuminous degeneration of the cytoplasm of most of the hepatic cells, but very little nuclear changes are noted.

The kidney shows no specific changes. There is a moderate granular degeneration of the tubular epithelium and a few small scars, apparently of vascular origin, just under the capsule, in which atrophied tubules and glomeruli are found. There is slight sclerosis of the large and medium sized vessels. The capillaries are all engorged with blood.

The heart shows some fragmentation of the muscle fibers, and slight edema in the connective tissue stroma, but the fibers stain well.

The adrenal glands show cloudy swelling and congestion and atrophic changes, consistent with the age of the patient.

Smears of the fibrinous plugs found in the bronchi show a few Gram-positive diplococci, apparently staphylococci, most of which are phagocytized, and a few short chains and pairs of Gram-positive cocci. The cells are chiefly polymorphonuclear leukocytes with a moderate number of large mononuclear cells. Giemsa stain shows no bodies recognizable as *Rickettsia psittaci*.

Sections of the lungs, liver and spleen stained by Giemsa are also negative for these organisms.

Gram-Weigert stains of the lung show rare diplococci in the exudate of some of the bronchi, but not in the parenchyma.

Mice injected with material from the lung, liver, and spleen developed typical lesions of psittacosis. (Karl Meyer).

Anatomical diagnosis

Psittacosis, with pneumonic consolidation of the greater portion of both upper and of right lower lobes; subacute splenic tumor; parenchymatous degeneration and dilatation of heart; generalized acute passive congestion; slight bilateral serous thoracic effusion;

slight benign adenomatous hypertrophy of prostate; moderate atherosclerosis of aorta and its larger branches; thrombosis of periprostatic veins.

SUMMARY AND CONCLUSIONS

The principal finding in fatal human cases of psittacosis is a unique pneumonia, involving diffusely the greater part of all of one or more lobes, producing a wet, consolidated parenchyma which is smooth and non-granular on section. The pleura, bronchi, and bronchioles are relatively spared. Histologically the consolidation is found to be due to an exudate consisting of fluid and varying amounts of fibrin, in the meshes of which large numbers of large mononuclear cells are found. Proliferation of the alveolar epithelium and histiocytes in the alveolar walls is a constant finding.

Moderate splenic tumor and congestion and cloudy swelling of the parenchymatous viscera is the rule.

Edema and congestion of the brain and leptomeninges is constant, ring-shaped hemorrhages in the cord and brain have been found in some cases, but thorough neurologic study should be made on all cases, since delirium and psychosis are clinically found in the majority of cases.

Post mortem diagnosis should be confirmed by inoculating white mice with material from the lung, liver, and spleen. Ante mortem diagnosis is usually not possible before the patient recovers or dies but can sometimes be made by inoculation of a series of white mice intraperitoneally with the patient's blood, or better with the sputum.

Since this paper was submitted for publication, a comprehensive report by Dr. R. D. Lillie has been published under the title: "The pathology of psittacosis in animals and the distribution of *Rickettsia psittaci* in the tissues of man and animals." It is published as National Institute of Health Bulletin Number 161. Most of the American cases are discussed and Dr. Lillie has examined the sections of these cases.

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A STUDY OF HYPERPYREXIA REACTION FOLLOWING INTRAVENOUS THERAPY*

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Hospital, Indianapolis, Indiana*

Hyperpyrexia associated with chill and fever following the intravenous administration of fluids has been a disturbing factor in this method of treatment. Despite the work done by able investigators along this line, the problem has not been solved. In this investigation it was found that the existence of these fever-chill reactions were much more prevalent in hospital systems throughout the United States than the authorities of each hospital were prone to admit. Various methods have been devised by different institutions to eliminate the occurrence of these manifestations with varying degrees of success. It was discovered that the prevalence of the reaction was looked upon as a factor which should be withheld from the report of the hospital and it was difficult to elicit exact information as to the incidence of occurrence of this reaction in different institutions that were investigated. In some instances rather forceful measures were exerted to suppress any information which might be given out concerning the occurrence of reactions. From various sources, however, it was learned that the hyperpyrexia reaction did exist rather prevalently throughout all sections of the United States, even though much work had been done to educate physicians to the desirability of eliminating this reaction.

In reviewing the literature it is shown conclusively that Seibert^{6,7} and Seibert and Mendel⁸ solved the problem rather definitely, but the acceptance of this work and the application of these results to practical therapeutic measures has been slow. In view

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

of the definitely concise scientific conclusions based on accurate laboratory studies, it is regretted that an attitude of obstinacy on the part of therapists to accept these facts still exists. The tenacity with which the average clinician adheres to his theory of the cause of intravenous reactions, leaves much to be desired in the hope of further education all along these lines. Experimental procedures controlled perfectly, together with unquestionably correct results appear to be rejected. Around the whole problem seems to be a fog of confusion.

In the pioneer work, Fortune in 1911 reported investigative work which was scarcely heeded. More recently studies were continued by Wechselmann,¹⁰ Tanner,⁹ and Seibert and Mendel, but these results have apparently failed to convince the therapist. Few wish to acknowledge that the etiologic factor lies exclusively in the field of bacteriology, yet Mendel and Seibert in their many studies placed it without doubt in the realm of bacteriology.

In 1930 Rademaker⁵ gave a very elaborate report of the steps which are necessary to follow in order to eliminate any pyrogenic reaction. In more elaborate detail the Committee of the American Medical Association Council on Pharmacy and Chemistry³ returned a very detailed and extensive report on the etiology of intravenous reaction, and followed this with recommendations for avoiding the occurrence of the chill and fever. In spite of these authentic recommendations they have not been generally accepted. Other investigators have gone further in the proposal of different types of tests in order to detect the presence of the substance which will here be referred to as a "pyrogenic substance." These tests have in the most part proved inefficient.

The one which was probably the most generally accepted and the most efficient was the one proposed by Carter.¹ This test seemed to carry unlimited promise, but upon extensive application in perplexing situations in which these reactions were occurring in showers, it was found to be totally inadequate. Upon study of the sensitivity of the test it was shown that even if the test as originally proposed was multiplied in sensitivity a hundred fold it would fail to indicate the presence of pyrogen in solutions which were heavily laden with this substance. The failure of

these tests to show the presence of the pyrogenic agent together with the failure of most individuals to follow the recommendations of the Committee of the American Medical Association has resulted in most elaborate proposals of etiologic agents. Essayists have advanced every conceivable factor which enters into the process of manufacturing intravenous solutions. There has been no definite indication as to which factor is most pertinent.

Little⁴ has called attention to every factor except the biologic one. In his discussion he has produced no evidence which might identify any one of these enumerated etiologic factors as the specific one. The entire problem presents a rather discouraging situation in any attempt to suggest or produce a satisfactory explanation that would be established by accepted scientific evidence. In view of the above mentioned situation this study was attempted to enhance and reinforce the data which had already been accumulated by previous workers. The study primarily was planned to substantiate or refute the numerous factors, which have been advanced as a causative, and to take into consideration and critically to analyze any test that had been advanced for the study of the detection of pyrogenic substance. The main problem divided itself into the following:

- (1) The range of pH of the intravenous solution administered. Are the extreme limits of pH responsible for the reactions?
- (2) The rôle the hypertonic and hypotonic salt solution play in the etiology of hyperpyrexia and rigor.
- (3) The manner in which particulate matter, such as lint, particles of sulphur from rubber tubing, dust and débris, contribute to these reactions as the causative factor.
- (4) The rôle temperature of the solution administered plays in the production of intravenous reaction.
- (5) The specific determination of the etiologic agent if possible.
- (6) The properties of the specific pyrogenic factor.
- (7) The value of proposed tests for the detection of the pyrogenic factor.

METHOD OF PROCEDURE

After careful analysis of all tests the only one thought worthy of consideration was the one devised by Carter. After frequent trials of this test it was found to

be totally inadequate and was soon discarded because it failed to give indication of the presence of massive quantities of pyrogenic substance in the solutions, even when the sensitivity of the test was increased a hundred times. The results were likewise uniformly false and this necessitated a consideration of another test.

In paralleling the work of Seibert and Mendel the normal rabbit was used and it was found in the injection of these animals that whenever the pyrogen was present in appreciable amounts, or even in minute quantities, the animal responded uniformly positively to the presence of this pyrogenic substance. The response of the animal to the intravenous introduction of the pyrogenic substance was prompt, usually within half an hour and continued through a period of three to four hours. When the substance was present in excessive amount the continuation of the reaction lasted as long as four hours. The return to normal was quite abrupt and usually once the temperature had begun to break it returned to normal within a period of an hour or so. The rabbit was selected because of the fact that the temperature range of the rabbit is normally from 101.5° to 104°F. It was noted that temperature conditions under which the animals were kept would also have an effect upon the temperature of the rabbit. When unusually high temperatures prevailed the animal temperature was slightly above that of normal, probably 0.1 to 0.2 degree, and in exceptional instances 0.5 degree. This necessitated keeping the external temperature conditions of the atmosphere constant in housing the rabbits. It was also noted that unnecessarily rough manipulation or exciting influences in the processes of handling the animals stimulated a rise in the animal temperature above that of normal range in a great number of instances. This obviously necessitated manipulation which would eliminate all such factors. With these conditions kept constant the results obtained from inoculation of the rabbit were sufficiently conclusive to indicate that the rabbit as an indicator was probably the most reliable and most dependable test object that could be used as an index for the presence of the pyrogenic factor in intravenous solutions. It was observed through this study that a few drops of water laden with the pyrogenic factor diluted to half a liter by pyrogen free water was sufficient to cause elevations of temperature in the animal to 105°F. and in some instances to 106°F. This solution was equal to a dilution of 1 to 1500, and in this dilution a maximum response was readily obtained. The volume administered was kept a constant factor in all studies. The volume was determined by attempting to parallel the greatest volume given at intravenous therapy following surgical operation and other medical procedures in man. If anything, the object was to exaggerate the conditions which might be produced in the human, in the attempt of therapy when the intravenous solution is given or administration seems indicated.

The first series of animals was given a constant volume of 12 cc. which was estimated to be about one-twelfth of the total available blood of an adult rabbit. It was also estimated that this was approximately in the same ratio of blood volume as the amount given the average human individual who received intra-

venous therapy following operative procedures. Any rise or elevation in temperature above that of the upper limit of normal of the rabbit was indicated as a definite positive response. That is to say a latitude of range between 101.5°F and 104°F was interpreted as normal fluctuations in the rabbit temperature. The variations which were found to occur in the animal's temperature between 101.5°F and 104°F were controlled with normal inoculated animals with known non-pyrogenic solutions. The ranges thus interpreted were so shown that there could not be any positive observations made in regard to the value of the normal fluctuation. After several hundred animals were inoculated it was quite easy to determine the irrelevance of the normal ranges.

TABLE 1

DEGREE OF GROWTH OF *Pseudomonas scissa* AND *Pseudomonas ureac* IN VARIOUS SAMPLES OF WATER

SOURCE OF WATER	DEGREE OF GROWTH
River.....	Heavy growth
Canal.....	Heavy growth
Creek.....	Heavy growth
Cold water main.....	Moderate growth
Hot water main (softened).....	Heavy growth
Well A (city).....	No growth
Well B (Riverside).....	No growth
Well C (J. L. J.).....	No growth
Cistern (J. L. J.).....	Slight growth
Well D (M. E. H.).....	No growth

The next problem of attack was to point out or to identify from the entire system of intravenous therapy the agent which seemed to be constant throughout all procedures and the one to which importance could be attached as the causative factor of reactions. One factor after another was eliminated as quite obviously excluded from the many factors that might be responsible for the intravenous reaction, until at last the bacteriologic or biologic factor was the one which definitely indicated the source of the fever-chill. The method of investigation was much similar to that used by Seibert and Mendel in the study of water bacteria. All sources of water supply from the city as well as private water supplies were studied from a bacteriologic point of view. In all instances which showed the presence of pyrogen by the rabbit test, these waters also showed the presence of growth of *Pseu-*

domonas scissa and *Pseudomonas ureae*, by cultural methods. In those waters in which cultures gave no growth, none of the members of the chromobacteria group could be identified nor could they be isolated after many attempts. In all of the specimens of water in which a severe pyrogenic reaction was obtained in the animal, a heavy growth of the organisms could be identified, either one or both species being present.

In table 1 the results of the bacteriologic study of various samples of water is shown and it is rather conclusive that water often carries the organisms and that they are not removed by modern treatment of flocculation and precipitation. It can also be seen that the artificial softening process of water as represented by Zeelite process does not remove the organism, nor does it remove the products of bacterial growth. Well water from the deep wells, deeper than 200 feet, is almost constantly free from any of these organisms. It is needless to state that other organisms are obtained from the cultures of water but the group of chromobacteria is entirely absent from deep well water. Cistern water shows irregularly the presence of bacterial strains of this group. Water obtained from various parts of the United States, from the South and the extreme North as well as the extreme East shows the presence of chromobacteria. These samples were obtained as a result of investigations in regard to the probable source of pyrogenic reactions which might come from ampule solutions. In all instances the ampule solutions were entirely blameless and the water from the supply of the hospital always showed the presence of these organisms.

The question was raised as to whether or not organisms of non-pathogenic variety would produce temperature reactions in the rabbit similar to the pyrogenic organisms. Likewise organisms with pathogenic classifications were suggested as possibilities of producing the pyrogenic response. A study of solutions containing killed suspensions of non-pathogenic organisms and pathogenic organisms were given intravenously to animals. From the group of pathogenic organisms *Staphylococcus aureus* was selected; from the group of non-pathogenic organisms common to water, *Bacillus subtilis*. For control a suspension of pyrogenic organisms was

used in the same study. A volume of fluid (pyrogen free water) was used as diluent. A constant volume of 12 cc. of each suspension containing fifty million organisms was the intravenous dose for each animal in this comparative study. The temperature of the animals were recorded for four hours following the injection. It was found that the pyrogenic organisms precipitated an immediate rise in temperature, with a prompt return to normal temperature level after two hours duration. In contrast the pathogens and the non-pathogens used, produced a delayed temperature response which followed an incubation period of an hour to several hours in duration. The return of the temperature to normal was gradual and prolonged over a period of several hours. The animal receiving the pyrogen showed relatively slight intoxication and after two hours of dyspnea and drowsiness was quite active and alert. On the other hand the animals receiving the non-pathogenic and pathogenic organisms were remarkably prostrated and remained so six hours or longer after the pyrogen was active. The specific property of this chromobacterium seems to be that of fever production.

The organisms isolated from the various sources of water supply which possessed the pyrogenic property were easily identified and grew best at room temperature or slightly below room temperature, 25°C. being the maximum temperature at which they grew luxuriently. These bacteria were chromogens and on dextrose agar grew luxuriently with the production of greenish or pale greenish-yellow fluorescence; they were gram negative and actively motile. They were small, plump, rod shaped bodies, about 1 to 2 μ in length, and ends of the bacilli were slightly rounded. These organisms were about 0.75 μ in width and in some instances almost simulated cocci. The growth was hazy in outline, particularly of individual colonies, rather serrated when examined under low magnification. The broth culture showed a very turbid growth with a light pellicle formation and this appeared at the end of about forty-eight hours. On potato good growth developed and was elevated with little tendency to spread; a very pale pink color was suggested. In litmus milk the reaction was slightly alkaline, no coagulation or digestion being noted. In gelatine stab there was a good growth but no liquefaction. Nitrates showed a slight reduction at the end of forty-eight hours. Up to this point the two species of organisms could not be separated. At the end of ninety-six hours indol was formed by one species, and not by the other.

When these two species were studied in regard to their fermentation of sugars,

the following results were obtained: *Pseudomonas scissa* produced no acid or gas after seventy-two hours in glucose, lactose, maltose, saccharose, raffinose, manite, inulin, and salicin. In levulose it produced slight acid after twenty-four hours but no gas after seventy-two hours. *Pseudomonas ureae* produced no acid or gas in glucose, levulose, lactose, maltose, raffinose, manite, and inulin,

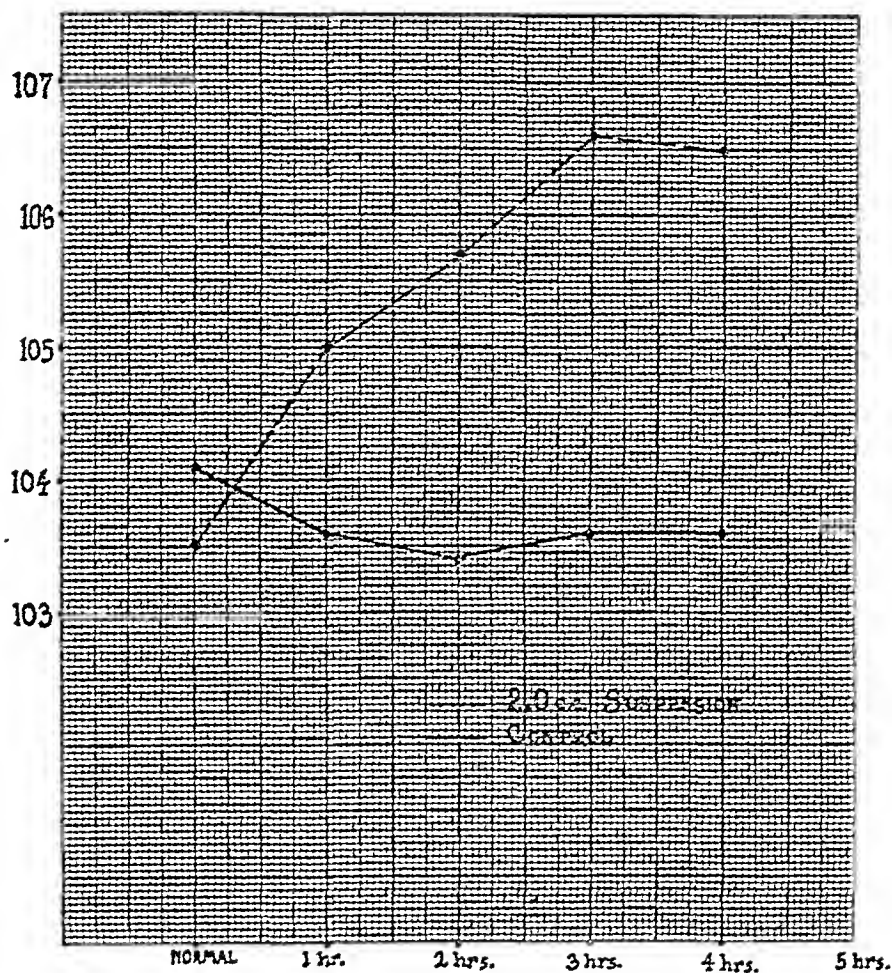


FIG. 1. REACTION FOLLOWING INJECTION OF WATER CONTAINING *Pseudomonas scissa*

but after seventy-two hours produced a very slight acid with no gas in saccharose and salicin. They were classified by following Bergey's Manual of Bacteriology.

Both of these organisms, as well as being isolated from the raw water supply, quite frequently were isolated from intravenous solutions, a part of which had been given a patient and had pro-

duced an intravenous reaction. Studying these organisms together with the ones which we derived from the raw water supply, the two species have been identified and frequently the same species was obtained from different sources. Each of these

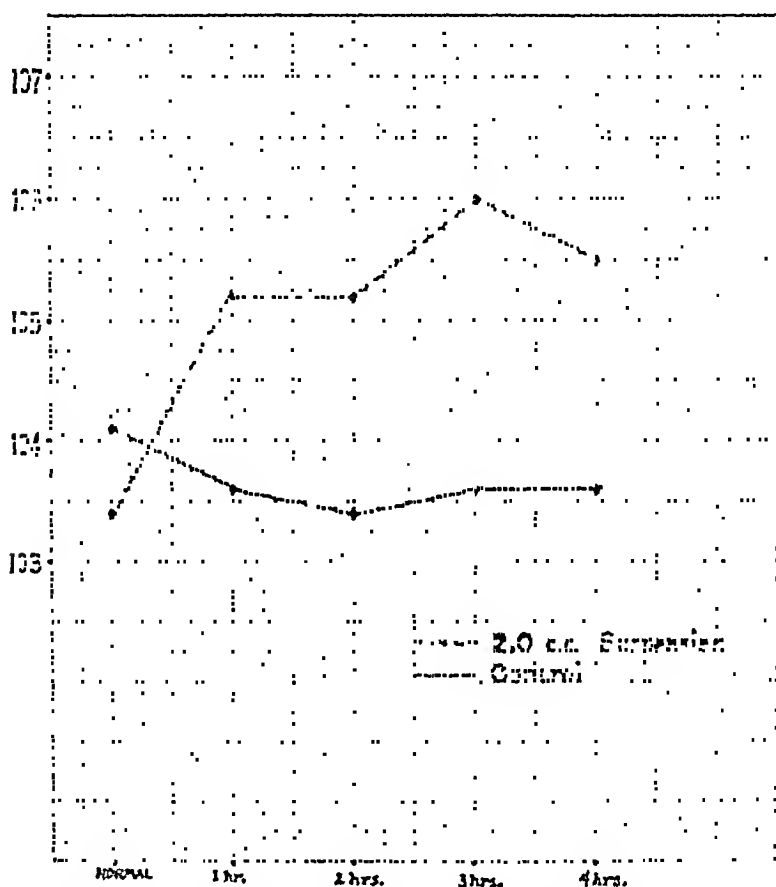


FIG. 2. REACTION FOLLOWING INJECTION OF WATER CONTAINING
Pseudomonas urcae

organisms produced approximately the same response in rabbits. (See figs. 1 and 2.)

In comparison to the other organisms which were isolated from the water, which were identified as not belonging to the chromobacteria, figure 3 shows that the pyrogenic response is absolutely

specific to these *Pseudomonas* strains. In the pyrogenic organisms the temperature response is very prompt in the first hour. There is very little or no period of incubation and a rapid return to normal. In the injection of non-pathogens there is a period of

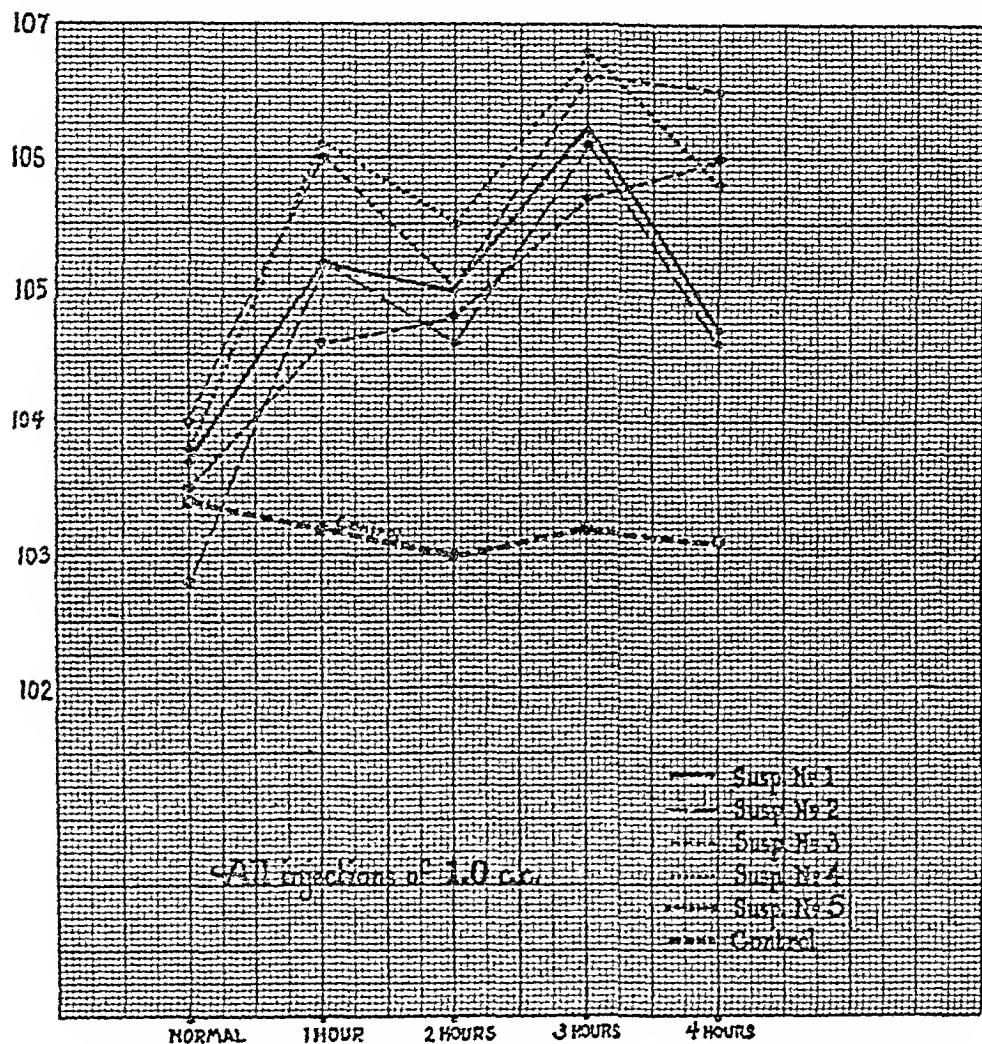


FIG. 3. REACTIONS FOLLOWING INJECTIONS OF PYROGENIC TEST SUSPENSIONS

incubation of an hour or two hours before a temperature response in the animal occurs and then the febrile period runs considerably over an hour or several hours, gradually returning to normal. With the pyrogenic organism the temperature return to normal is very abrupt after a period of several hours.

In the light of these studies it seems justifiable to attribute to these strains of organisms the property of specific pyrogenic activity.

The next procedure was to identify the rôle of other factors in the production of intravenous reaction when the presence of the pyrogen had been quite definitely excluded. In the attempt to accomplish this object pyrogen free water which had given no temperature response in injection of the animal, was used. Sodium chloride was added to this water to make a normal saline solution containing the specific organism. It was then determined whether or not the pyrogen producing substance was a portion of the bacteria themselves or was a substance which was produced as a result of bacterial growth. It was difficult to believe that the growth of organisms occurred in the distilled water, practically devoid of all nutrient materials, which are considered as necessary to bacterial growth.

Using the same constant conditions of the previous studies on rabbits the preparation of killed, bacterial suspensions was diluted with pyrogen free water, so that a suspension of 250 million organisms per cubic centimeter was obtained. Two cubic centimeters of this suspension were given intravenously. The bacterial suspension gave prompt response and a high temperature reaction which lasted over a period of several hours, returning abruptly to normal. An occasional instance was noted in which the animals experienced a severe rigor associated with the temperature rise. Bacterial free filtrates of dextrose broth upon which the organisms were grown were used as material for injection after filtering through Berkefeld filters. The filtrate was controlled as to growth of organisms. In all instances where the controls were negative at a period of forty-eight to ninety-six hours doses of these solutions were given. The uninoculated broth cultures were used as controls. Doses of over 5 cc. of the broth filtrate gave slight temperature reactions of about 0.5° above 104°F . The temperature developed from both types of substances was parallel, the temperature response from the bacterial suspensions was markedly more severe than that obtained by the bacterial filtrates. The substance within the bodies of

the bacteria themselves seems to carry more severe reactions than those substances which are produced extrabacterially. A more recent study has shown in the work on filtrates from the pyrogenic bacteria that the amount of pyrogenic substance generated exobacterially is one twentieth as concentrated in the production of the temperature response as the endobacterial substance. From the above studies it would seem that the pyrogen factor specific to these strains of organisms is an endotoxin rather than an exotoxin, and comes about as a result of the biological composition of the bacteria themselves rather than from the products which are liberated into the media in which they may be present as a result of bacterial growth.

EFFECT OF HEAT WITH AND WITHOUT PRESSURE

A rather interesting development occurred in the process of sterilization of these bacterial suspensions. Inactivation at 58 and 60°C. for one hour were used on some suspensions while on others autoclaving was used, at 20 pounds pressure of steam.

In vaccine work it is generally conceded that the autoclaved preparation is practically valueless and the inactivated preparation of vaccines are considered the most efficacious. It may be seen by figure 4 that in the heated preparation at 58 to 60°C., the temperature response began in about one to one and one-half hours following the injection. In the autoclaved preparation the temperature response began promptly inside of half an hour. The autoclaved product yielded a temperature of 1.5 to 2° higher than the heated product. The autoclaved product was also carefully controlled in regard to the efficiency of the autoclaving and no forms of bacteria could be morphologically identified after this autoclaving process. It is rather interesting to observe in view of the fact that the process which yields the most severe temperature response is based on a process which is used almost universally in sterilization of intravenous solutions. This would seem to indicate that if the solution which is to be used for intravenous work contains any pyrogenic element, the sterilization which is now employed as routine in most hospitals will activate or enhance the possibilities of an intravenous reaction in these

solutions, which otherwise might be eliminated or be considered as latent.

In view of the problem of storage of intravenous solutions in the hospital, the question arose as to whether or not a pyrogen free

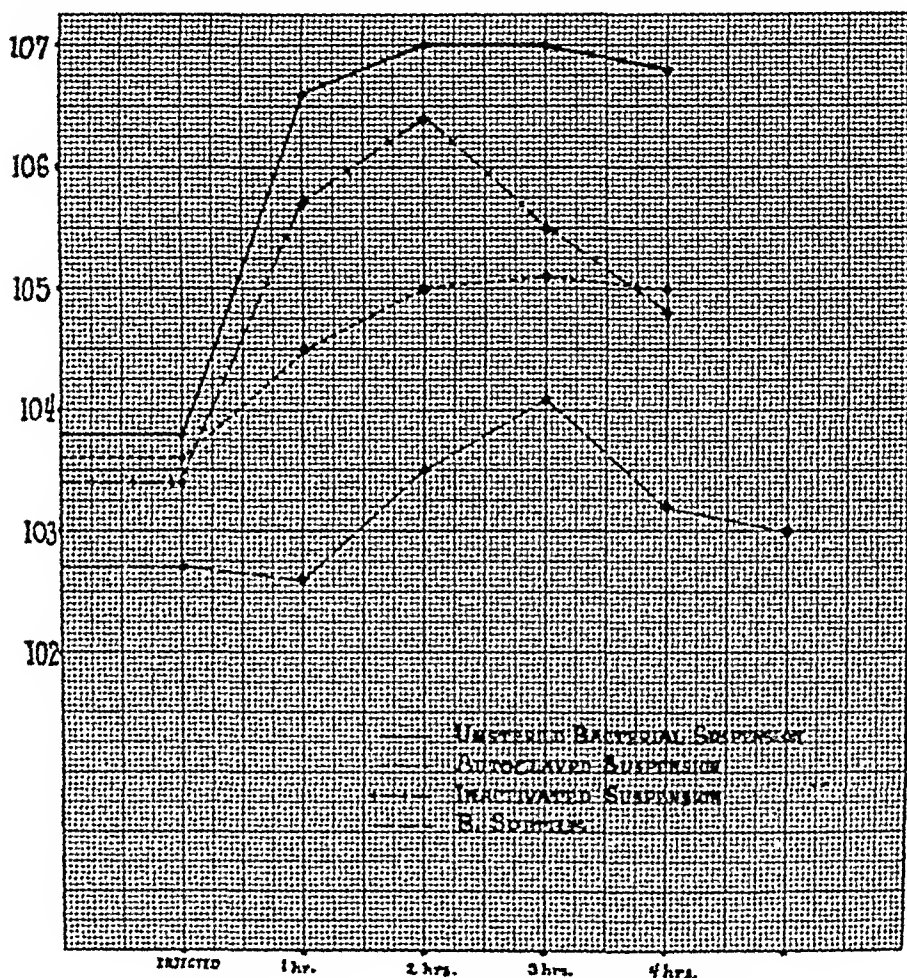


FIG. 4. EFFECT OF TYPES OF HEAT ON PYROGENIC SUBSTANCES

solution might become pyrogen laden upon storage. There also arose the question as to whether or not the pyrogen laden solution increased in its degree of pyrogenic activity upon storage. Under the same conditions relating to the animal experimentation both

pyrogen free water and pyrogen free intravenous solutions were stored. At the same time definitely known pyrogen laden water was stored without any attempt at sterilization or any attempt at careful sealing. At irregular intervals throughout a period of ten months these solutions were injected into animals and observations made as in the first experiment. The results obtained were rather interesting in that the temperature response on the first administration of the contaminated water was maximal and the subsequent injections of the same solution at irregular intervals over a period of ten months gave the same responses. The response to a constant volume showed the same maximal intensity of reaction, that is to say, the first injection of raw water was parallel to and almost exact in all of its details to the reaction which was obtained on the last administration. The pyrogen free saline was stored, together with the water samples found to be pyrogen free. After this long period, on subsequent injections these solutions did not show any pyrogenic activity. The solutions of intravenous type were quite tightly sealed. The water specimens from different sources were not sealed with any more than the usual stoppering.

This study indicates that non-pyrogen bearing water when sealed under average precautions does not become pyrogen laden upon storage. Likewise the same may be said of intravenous solutions which are hermetically sealed; these will remain pyrogen free as long as a period of ten months. It would seem unjustifiable to place the blame for intravenous reactions upon either freshly or stored distilled water when these substances are kept under reasonably careful conditions. It was pointed out by Seibert and Mendel that these strains of organisms grow in distilled water. From such observations as have been obtained it would rather seem that they do not grow in distilled water but are introduced through a process of handling. Once present this substance remains in the water and is difficult to eliminate except through a very efficient distillation process. It may be that freshly distilled water is a medium for growth of these pyrogen producing organisms, but from observations which have been obtained this statement is only true to a certain degree. One qualification

must be fulfilled in order that the growth may take place, that is, that distilled water must be contaminated coming from the still or contaminated in or after collecting.

The pyrogen free water if hermetically sealed remains so indefinitely, stored at any temperature. In view of this fact, properly made intravenous solutions which are known to be pyrogen free will remain so, whether stored one day or one year. The still or the vessels contaminated by the pyrogen are easily freed of this agent by thoroughly washing in distilled water.

SOLUBILITY OF PYROGENIC SUBSTANCE

The next problem in the process of study of this particular factor as applied to use in hospital routine, was in regard to freeing containers which might be contaminated.

If the containers be contaminated what steps are necessary to free the containers of this substance? In other words, is the pyrogenic substance soluble in water, and will mere washing of the contaminated vessels with pyrogen free water eliminate the substance? This is very clearly demonstrated in handling the glassware and also in handling the still. At periodic intervals the still, if not carefully watched or equipped with a spray trap, will bubble over and the pyrogen contaminated substance will be carried into the distilled product. It was found that flasks which contained the pyrogen bearing substance were universally freed of the offending substance by frequent washing with pyrogen free water, say three or four times. It is of little consequence whether or not the pyrogenic laden water or solution has been evaporated upon the surface of the container as it is readily soluble after being dried. Should the still be contaminated by dismantling for repairs or other purposes a run of two hours properly manipulated is sufficient for completely freeing the still of this contamination. In case of discontinuing the operation of the still, it is always safest to run the still for at least one hour before any water is collected for intravenous use. It has been found by frequent examinations that this length of time almost constantly precludes the possibility of any contaminated water. If, however, the water is collected immediately upon beginning

operation of the still it is almost certain, even if the most extreme care is used, to produce a pyrogen laden product. (See fig. 5.)

A study was made to determine the effect of sterilizing water for varying lengths of time in order to learn if pyrogenic substances could be eliminated by this process. It did not appear that ordinary sterilization as routinely practiced would remove the substance. If the finished intravenous solution produces a fever reaction after it has been sterilized for one-half hour at 20 pounds steam pressure after being sealed before and remaining

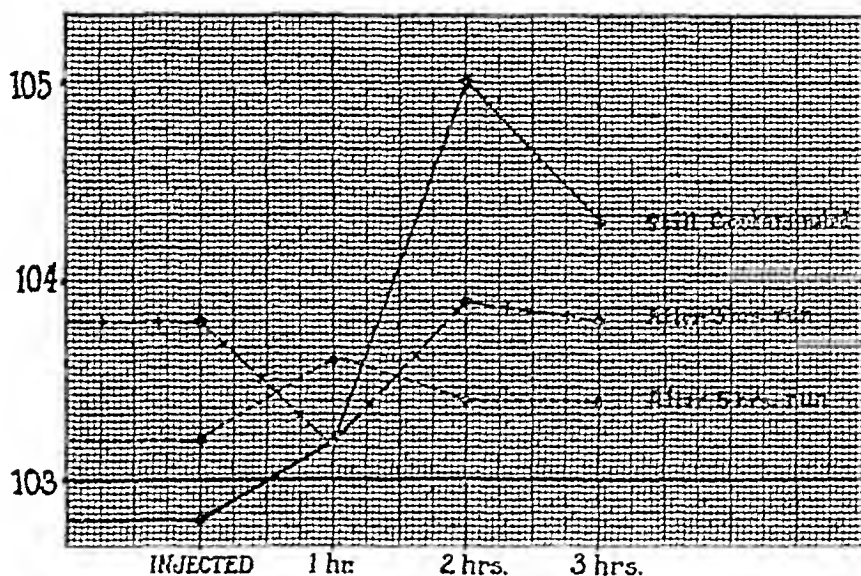


FIG. 5. EFFECT OF PYROGEN CONTAMINATED STILL ON DISTILLED WATER

sealed after sterilization, there must be some factor that is resistant in the fever producing substance to ordinary sterilization ranges. In this study rabbits were used as in the preceding studies and the same volume of fluid was used. The animal which was used as a control was given pyrogen free water of the same volume at the same time. This pyrogen element which was artificially introduced into the pyrogen free controlled water was obtained by using a bacterial suspension of *Pseudomonas scicca* or *ureae* in autoclaved preparation. The seeded solution was injected and produced very prompt rise in temperature in the normal rabbit. The response lasted over a period of time rang-

ing from two to five hours. When one autoclaves this mixture at 20 pounds pressure for one-half hour the response is more prompt than one obtains in the original preparation without autoclaving. At the end of one hour the sterilization of the pyrogenic factor is not

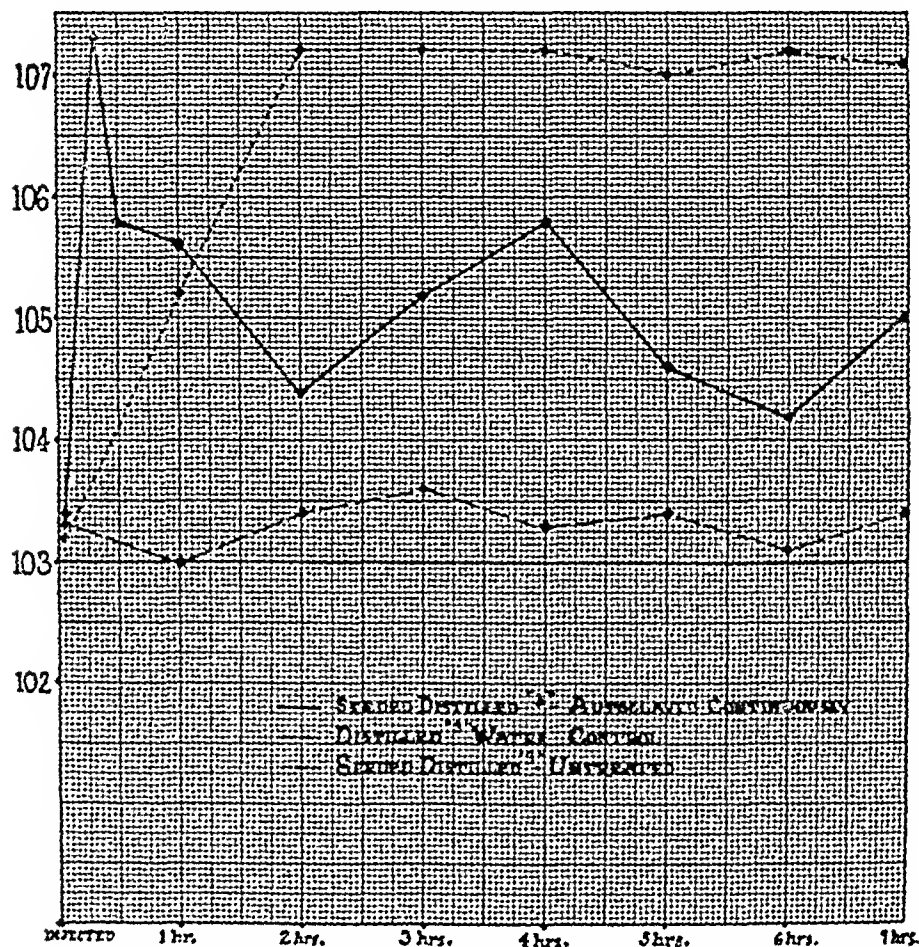


FIG. 6. DISTILLED WATER SEEDED WITH PYROGENIC ORGANISMS AND AT A PH 6.4

decreased. At the end of two hours sterilization there is a slight decrease in the pyrogenic response. At the end of the fourth hour the pyrogen begins to disappear and the febrile reaction is quite distinctly lessened but not until the sixth or the seventh hour does the pyrogenic factor completely disappear and no tempera-

ture response is elicited. Figure 6 seems to convey a justifiable conclusion that ordinary sterilization has no effect in destroying the substance which produces the fever-chill reaction, but seems to enhance or stimulate this activity.

In some institutions investigated it was found that the intravenous solution prepared within the hospital itself showed it to be practically free from pyrogen in all instances. In these institutions it was found they used the same common source of water supply as the majority of the other hospitals. The point of departure in their routine lay in the fact that they used a distillate from the circulating steam, carried at 60 pounds pressure through the mains. This was condensed into water and condensed steam conducted through a distillation process. In the course of circulation through the mains of the high pressure steam system a temperature of 140°C . was maintained throughout the entire system. The pyrogenic factor would be easily broken down at this temperature in a short period of time. It was rarely found that the condensate could be collected before half an hour to an hour's circulation through the system. Thus it seems that from this practical application it is easy to demonstrate that the substance causing the fever-chill reaction is readily destroyed and completely broken down at a temperature of 140°C . in thirty minutes.

HEAT RESISTANCE IN ALKALI MEDIA

In view of the fact that many competent laboratory directors recommended the addition of alkali in sufficient quantity to distilled water to produce a pH of 7.6 when glucose was added to the water, this study was made in order to show the effect of sterilization upon such a mixture. The tests were done with controlled pyrogen free triply distilled water to which was added sufficient alkali to produce a pH 7.6 when glucose was added. This solution was also carried higher in alkali content until the pH was 8.4; and after sterilization this reached 9.2. These alkali solutions in the ranges of pH 8.4 to 9.2 in controlled pyrogen free water were injected intravenously in the animal, and there was no elevation in temperature whatsoever. Pyrogen free solution of pH 8.2 does not give an elevation of temperature. (See fig. 7).

Repeated injections of these solutions confirms the above statement. It was plainly seen that alkali alone added to the distilled water had no influence on the temperature zone. This same degree of alkali was added to the pyrogen inoculated water and it was found that the temperature curve of the animals showed as prompt, as maximal, and as rapid a response as the pyrogen laden distilled water to which no alkali had been added. Consequently

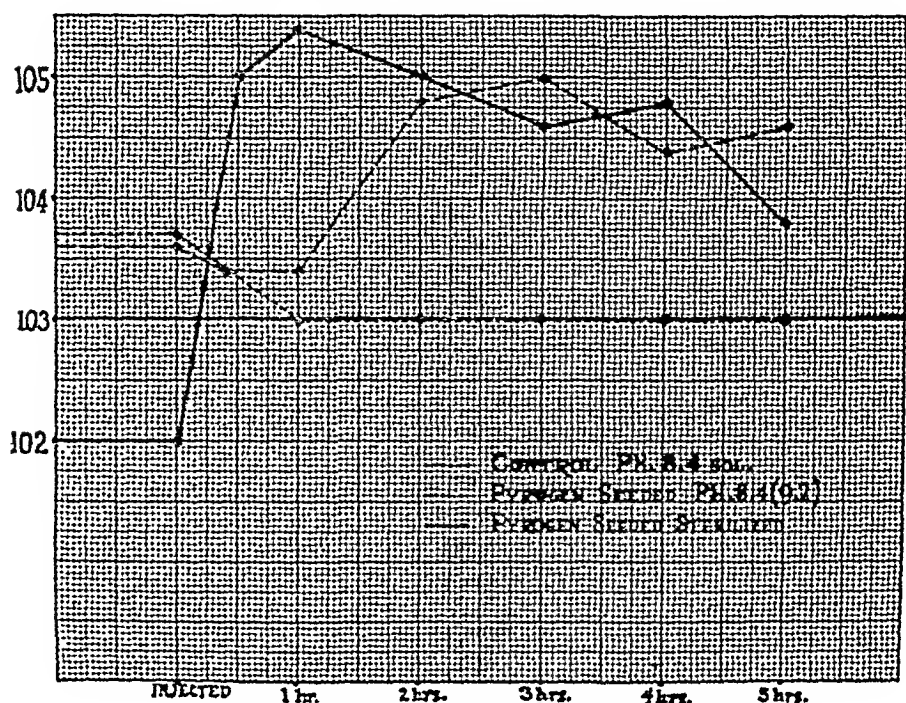


FIG. 7. EFFECT OF ALKALI ON PYROGEN

the mere addition of alkali in the pyrogen laden water had no effect whatsoever. This condition produced artificially by the addition of alkali was added to the influence of sterilization.

It was thought possibly that the heat element might have something to do with the destruction of the pyrogenic factor after the alkali was added or at least caused the pyrogen to disappear more rapidly when subjected to sterilization. In view of this hypothesis the following study was made.

The same constant conditions were produced as in the other studies and the same constant volume was introduced into the circulation of the rabbit with observations every hour for three to four hours. The response was much the same as in the pyrogen contaminated water, except that there was a slight delay in the reaction of the febrile response. The sterilization at 20 pounds showed that in order to have any effect upon the pyrogenic substance four hours continuous sterilization must be employed but the 5th hour of sterilization guarantees complete safety. Thus, alkali itself does not change the character of the fever-chill reaction when added to pyrogen laden distilled water and likewise addition of alkali and sterilization for one hour does not influence the breaking down of this element.

HEAT RESISTANCE IN EXCESSIVELY ACID MEDIA

A contrasting study was made in excessively acid media. In both of these studies it was not attempted to produce a pH in either range that would be intolerable to man if given intravenously, because outside of this range it would be practically impossible to adopt any such measures by the addition of acid or alkali to any solutions that would be able to be utilized in human therapy.

The controlled mixture of pH 3.8, showed on its administration (fig. 8) when made up with pyrogen free water no evidence of fever-chill response. When pyrogen was added the response was quite active, very prompt and considerably increased above the level demonstrated by neutral solutions. With the addition of pyrogen to the pyrogen free solution in a medium of pH 3.8 the fever response was quite prompt. This rise appeared within half an hour after the injection and continued so for three to four hours, then abruptly receded to normal. When this mixture was submitted to 20 pounds pressure of steam for one half hour very little effect was shown upon the maximal response except the fever-chill reaction occurred much more promptly than in the unsterilized mixture. At the end of one hour however, the pyrogen substance showed marked decrease after being subjected to sterilization. The response was very slight as compared to the

original mixture. At the end of two hours the pyrogen was completely destroyed and no febrile response could be elicited from the solution.

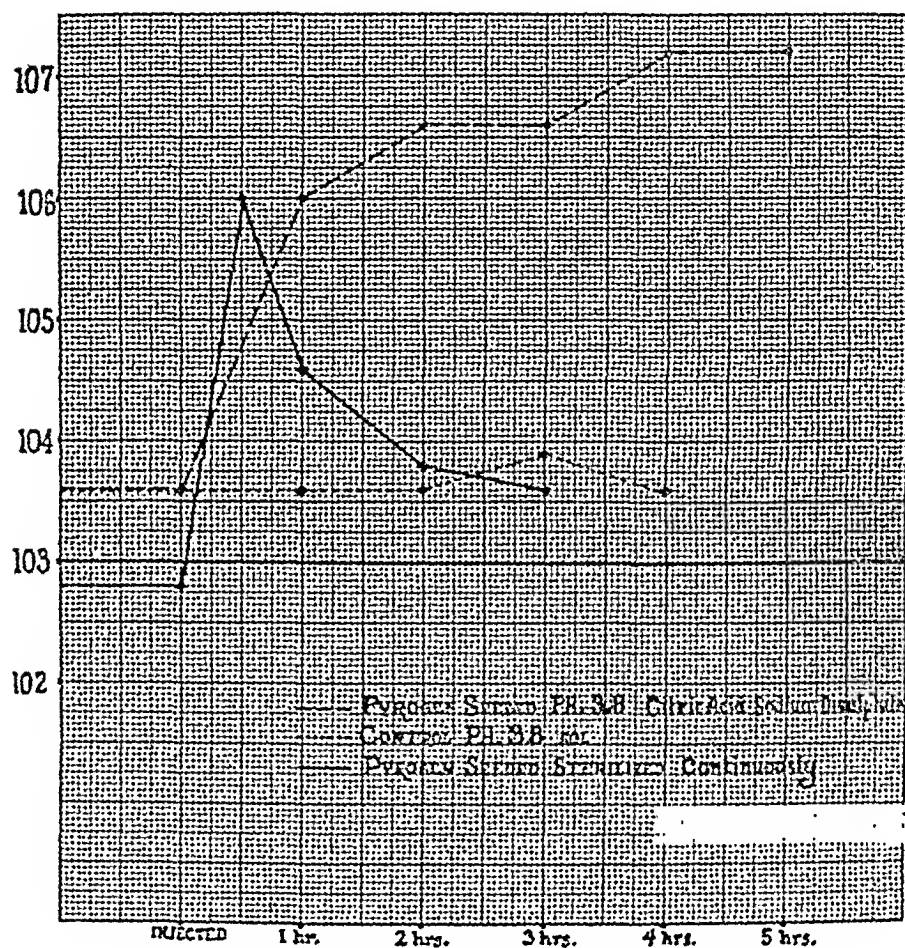


FIG. 8. EFFECT OF ACID ON PYROGEN

HEAT RESISTANCE IN NEUTRAL MEDIA

Up to this point the determination of the resistance of the pyrogen element to heat had been conducted in media which was either of the pH of distilled water or of the excessively acid range or excessively alkaline range. The pH of normal saline which is

usually available from the water of triple distillation results in a pH of 7. Pyrogen free saline was treated with a definite known quantity of bacterial suspension of these chromobacteria. This on being introduced into rabbits in the same volume as that which has been described in the previous studies showed a remarkably prompt response paralleled in a great measure by the same type of temperature reaction that one gets in the excessively acid or in excessively alkaline solution. There is one difference, however, and that is the reaction with the normal saline medium gives a more prolonged temperature response than with either the acid or the alkali. The resistance of the pyrogenic substance in normal saline when exposed to heat, 20 pounds pressure, shows a sluggishness in breaking up. The fever-chill reaction substance is much more active in the solution sterilized for one hour than it is unsterilized, and there is no decrease in the maximum response after the substance has been treated for four hours consecutively at 20 pounds pressure. After the fourth consecutive hour there is an appreciable decrease in the fever chill reaction but it is not marked. There is a gradual decrease in the amount of reaction until the seventh hour is reached and then the fever-chill reaction entirely disappears from the solution.

HEAT RESISTANCE IN DISTILLED WATER

In a similar study the pyrogen-free distilled water was controlled and seeded with the culture of these pyrogenic organisms (fig. 6), and then subjected to sterilization of 20 pounds pressure of steam for each consecutive hour until the substance showed by animal inoculation no response in the fever-chill reaction. In this triply distilled water the reaction is much the same as that which is experienced in the other ranged of pH, regardless of the solute that is placed in the water. The duration of the temperature reaction of the animal is approximately the same as in the neutral solution. When subjected to 20 pounds steam pressure it requires seven hours for it to be completely destroyed. This is quite analogous to the result which is previously obtained in the study of the reaction of the normal saline. Up to four hours continuous steam sterilization of 20 pounds the pyrogenic substance

is not affected, it is quite comparable to the normal saline. At this point there is a perceptible lessening of the intensity of the reaction and from this period of sterilization up to the end of the seventh hour there seems to be very slight change.

Comparison of the graph of the resistance of the pyrogenic factor to steam sterilization shows that in excessively acid media the pyrogenic factor is destroyed in a shorter period of sterilization than any other range of pH. Neutral range shows the least effect on the destruction. The alkaline range shows a definite influence in increasing the rapidity of destruction but the time factor necessary for the destruction of this agent is definitely longer than that of the acid ranges. The theory that the addition of sufficient alkali to readjust the pH of distilled water to neutral after the addition of any pH influencing substances destroys the pyrogen must be considered as erroneous. In the light of these observations it seems justifiable to conclude that the accepted procedures of sterilization of intravenous solutions activate the pyrogen and enhance the chances for reaction, if the water used for intravenous solutions is pyrogen laden. Also the addition of either acid or alkali to pyrogen laden solution shows slight effect on its pyrogen content. The substance is not destroyed or rendered inert by the addition of acid or alkali which seems to increase the severity of reaction. The addition of acid or alkali to a limit tolerated intravenously by living individuals does not cause more rapid dissolution of this factor when exposed to 120°C. for seven hours. This is much too long a sterilization period to be practical and higher temperatures must be used.

In the work done here the results show that 145°C. for one-half hour completely destroys all pyrogenic substances of either the bacterial suspension or bacterial filtrate. In corroboration of this work it has been shown that institutions which utilize condensed steam from their heating plants or the exhaust steam of these plants for distillation have few reactions. This process destroys the pyrogenic factor in the water before being brought to the still. Under this system described, the occurrence of intravenous reaction is inexcusable.

RÔLE OF HYPERTONIC AND HYPOTONIC SALT SOLUTIONS

In further study of the pyrogenic substance the influence of hypertonic and hypotonic salt solutions was investigated. The same factors were duplicated as in previous experiments and the same quantity of solution injected. The hypotonic salt solution made from pyrogen free triply distilled water, was of the strength of 0.4 per cent and the hypertonic salt solution, 1.2 per cent. The animals were injected as in the previous studies and figure 9 shows very slight response in temperature curves, the maximal

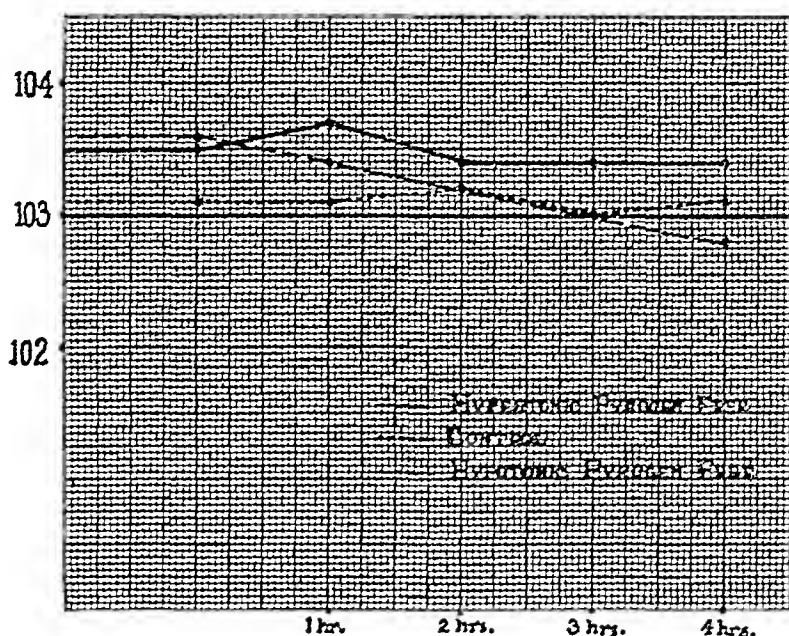


FIG. 9. EFFECT OF HYPERTONIC AND HYPOTONIC SALT SOLUTIONS ON PYROGEN

variation being a few tenths of a degree. The temperature never reached the upper limit of normal in using either solution. The slight increases in temperature were well within the limits of physiologic variations of rabbit temperature. A slight response demonstrated by the administration of these solutions indicated that little credence may be placed in the claim that either hypertonic or hypotonic salt solutions are responsible for the fever-chill reaction following intravenous therapy.

RÔLE OF VOLUME OF FLUID INJECTED AND RATE OF INJECTION

Another factor which has often been given as the cause of intravenous reactions is the volume of the solution which might be administered, regardless of biologic or chemical content. This idea is based on the theory that the volume of the fluid administered at one time might be so massive as to cause disturbance of the water equilibrium between the circulating fluids and the body

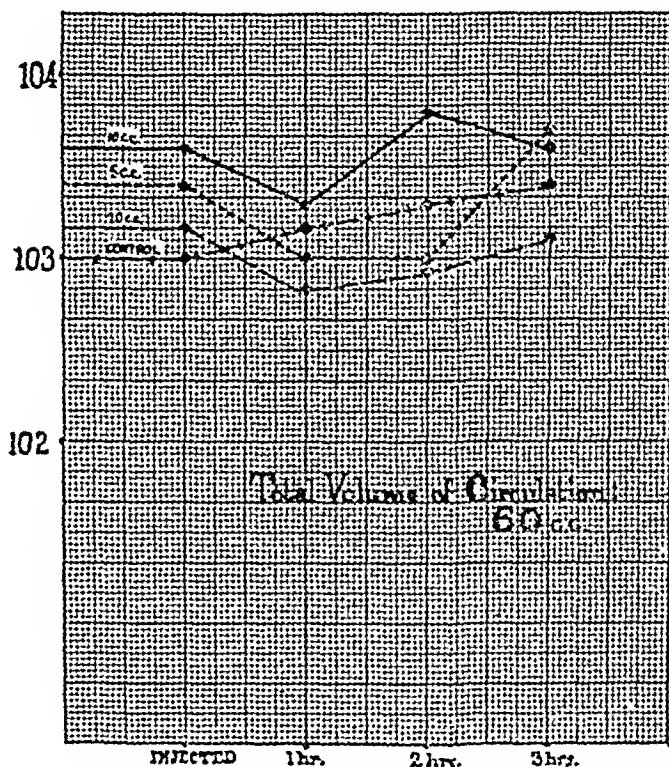


FIG. 10. RELATION OF VOLUME INJECTED TO REACTION

tissues, causing a fever-chill reaction. So popular is this idea among individuals who resort to intravenous therapy that very intricate and elaborate machines have been devised to administer to man large quantities of fluid in small doses over a long period of time. These machines have been so devised as to place a cannula in the veins permanently and to allow through accurate measurement a small amount of fluid to be introduced into the

circulation at regular intervals, thereby eliminating all possibilities of intravenous reaction, the volume itself being the main factor that is considered in the administration of the fluid. No other factor has been taken into consideration in this particular phase of therapy by intravenous route other than the volume. The content of the fluid has not been critically analyzed as to the possibility of what might be causing a reaction.

In the experiments to test this hypothesis the volume of the fluid alone was considered. Varying amounts from a few cubic centimeters to 24 cc. of both pyrogen laden and pyrogen free solutions were introduced. In all the pyrogen laden solutions the temperature response was quite prompt, and the characteristic picture of duration was obtained in all the studies. In the pyrogen free solution regardless of volume, the temperature response was not of abnormal variation, but well in normal limits and infrequently varied not more than one half degree (fig. 10). This variation in temperature is probably ascribed to physiological changes normally present in the individual animal under experimentation. Any volume of pyrogen free fluid itself will not cause a reaction but should the pyrogenic substance be present in the solution, whether the volume be small or large, the response will be unquestionably and unfailingly a fever-chill reaction. During this study the rate of injection likewise was investigated and in all of the 937 animals, the rate of injection was varied at different intervals. In the last several hundred of the series the time of administration was limited to short time, the entire volume being introduced in the animal in the time that is required to rapidly empty the syringe through a 26 gauge needle. In all of these studies it was found that the rate of administration bore little influence to the temperature response of the animal.

IMPORTANCE OF TEMPERATURE OF SOLUTION

In the course of this study attention was called to the rôle that the temperature of the solution administered might play on the temperature of the individual receiving the solution intravenously. In experimental procedure in animals this was reproduced in the most radical extremes that could be encompassed in

the range of solutions administered to the human. Controlled pyrogen free solution was used and was administered to the animal in volumes of 12 cc. The temperature of the solution was then varied from 68°F. to 113°F. Figure 11 shows the influence of the range of temperature of solution.

One animal was selected in which the temperature was at the upper limit of normal. In this instance the temperature of the solution given was the same as the body temperature of the rabbit, that is to say, a temperature of 104°F. In this instance the temperature was elevated 0.2° above the normal range of the animal. The other solutions administered varied in temperature from

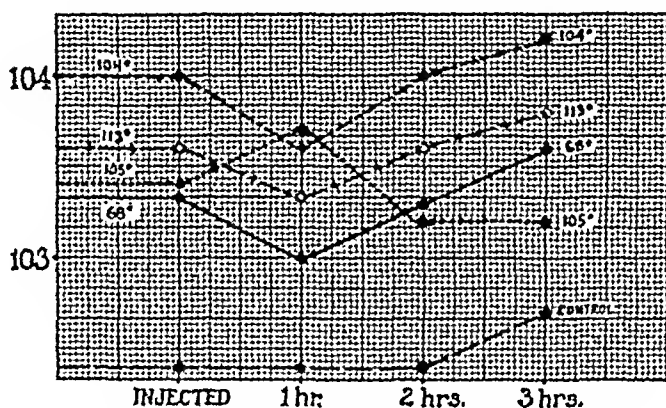


FIG. 11. EFFECT ON REACTIONS OF VARYING THE TEMPERATURE OF SOLUTIONS

68°F. to 113°F. The total range of fluctuation was about one degree. Never was the upper limit of normal of the rabbit's temperature reached at any time during the period of observation. This particular experiment was carried on under conditions in the mid-summer in which the animals were not kept in an excessively cool atmosphere. The same care was taken to provide the atmospheric conditions that would be usually associated with intravenous administration of solutions to the man. In order to deliver the fluid at the temperature which was indicated on the thermometer, the solution was injected through a syringe-needle, and introduced into the animal's circulation as rapidly as possible through a 26 gauge needle.

As to the immediate effects of temperature of the solution administered upon the individual receiving the solution little information or no definite conclusion can be drawn from this experiment. It would seem that the temperature of the solution administered has little influence upon the temperature of the individual receiving the solution. There might be possibility that a solution above body temperature might activate an otherwise latent pyrogen.

THERAPEUTIC APPLICATION

The pyrogenic agent was applied therapeutically through the collaboration of Dr. H. K. Langdon and the clinical application was done jointly with him.

In this study of practical therapeutics in the human individual, these bacterial suspensions were administered to a certain group of patients who had been previously treated by the administration of malarial plasmodia for production of hyperpyrexia. In a few cases this substance was administered to individuals who were rather advanced in central nervous system lues, dementia paralytica and tabes dorsalis, and the results derived were rather surprising (fig. 12). The temperature curve responded very rapidly in a parallel curve such as is derived from a malarial rigor, lasted from five to six hours, and then receded to normal. A rigor lasting from twenty to thirty minutes was experienced.

The blood count, contrary to that which is derived from a malarial inoculation, during the rigor increased from 90 to 100 per cent. The polymorphonuclear percentage of the leucocytes rose to about 95 per cent.

The condition produced as a result of the administration of this material to paretics is easily controlled. The reaction may be reproduced every day or twice during the day, or whenever the therapist so desires. The patient recovers from the rigor very promptly. There appears to be no dangerous manifestation and temperature ranges from 100.4° to 106°F. are easily obtained. There is no disease to combat after the rigor has passed, and the following twenty-four hours the individual is normal. A rather interesting manifestation which has developed recently in the

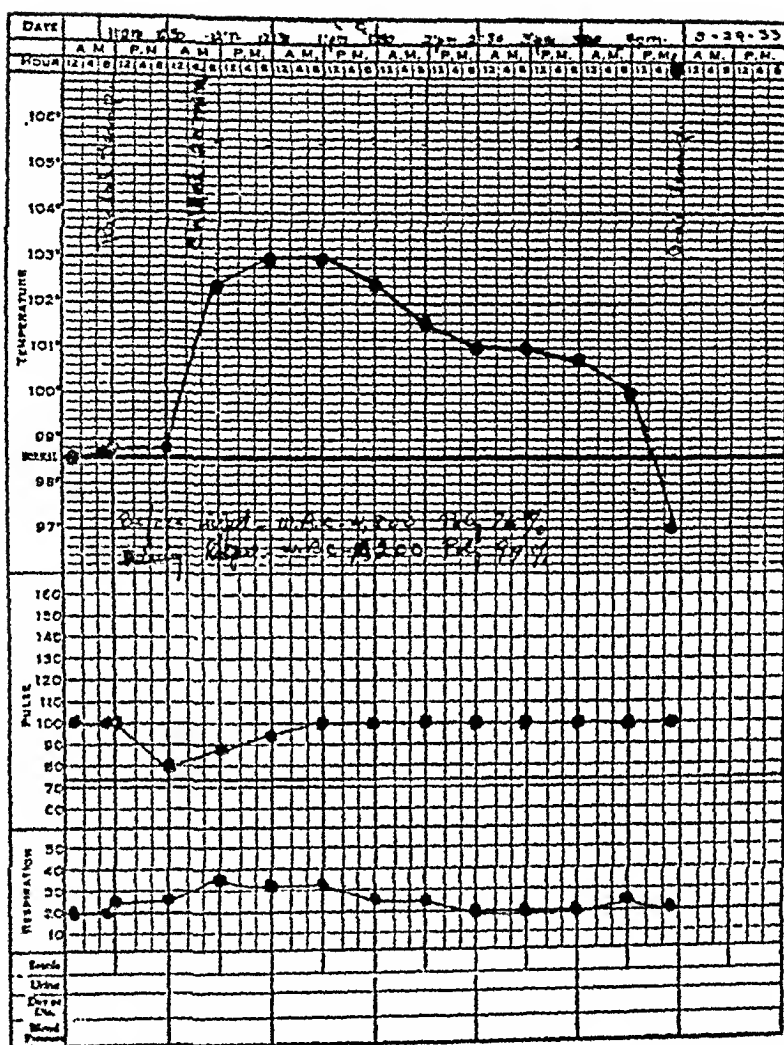


FIG. 12. TEMPERATURE CHART OF A PARETIC RECEIVING PYROGENIC SUBSTANCE FOR THERAPEUTIC EFFECT

Before injection the leukocyte count was 4,800, with 76 per cent polymorphonuclears, after the injection and during the reaction the leukocyte count rose to 10,200, with 94 per cent polymorphonuclears. The temperature was recorded every 30 minutes.

use of this agent is the fact that the hemoglobin and the erythrocyte count mounts steadily during the process of reaction and there is a definite increase in the number of reticulocytes. After

administration of two to three weeks the cerebral manifestations begin to clear up and psychically the patient becomes markedly improved.

The series is too small and the work incomplete so that details of serological changes and laboratory investigations other than that which we have just begun, are not yet available.

DISCUSSION

With the animal studies upon which these investigations were made, it was found that any chemical test as designed for the detection of pyrogen in water or in solutions was totally inadequate. The only test which would unfailingly and constantly show the presence of pyrogen substance in waters and pyrogen contaminated solutions was the use of animals. This test is reliable and without variation. An interesting feature about the use of the pyrogen contaminated solution in intravenous work in animals is that the animal does not become immune to the pyrogen nor is there any evidence that the animal becomes hypersensitive to the pyrogen. In other words, the thermogenic reaction was maximum with all doses of pyrogen whether repeated the same day or several days later. The response in all instances is practically maximum when different sized doses of the pyrogenic substance were given. All doses showed a more gradual reaction in the response, the slightest dose sufficient to give a reaction was 0.03 cc. of a stock solution regardless of what diluted volume was used.

The volume of the fluid injected has little effect upon temperature reaction, provided that the solution is pyrogen free.

The range of alkali and acid solutions from pH 9.4 to 3.8 show little effect in changing the temperature of an animal when large volumes are given, or when small volumes are given, provided that no pyrogen substance is present in the solution.

Particulate matter in dense concentration has practically no effect upon the temperature range of the animal if the water containing the particulate matter has no pyrogen content.

In the study of the effect of hypertonic and hypotonic salt solution upon the fever-chill reaction of the animal, there is no response if the solution contains no pyrogenic substance.

The temperature of solutions administered plays very slight or no influence in effecting the temperature curve of the injected animal. If solutions are used at a temperature above that of the normal animal temperature, it is observed that this temperature will activate an otherwise latent pyrogenic solution. Otherwise if the pyrogen is absent the temperature of the solution has no influence upon the temperature of the animal.

The substance causing the intravenous reaction is specific for that type of reaction. It is derived from the bacterial bodies and products of growth of *Pseudomonas scissa* and *Pseudomonas ureae*. These strains predominate in this reaction. Unless this substance be present in the solution, all other conditions intentionally produced cause no effect upon the temperature curve of the rabbit.

The specific property of this substance, that is, the pyrogenic property, has been shown to be effective for man as well as for rabbits. The pyrogen substance is water soluble and is rapidly eliminated by frequent washings with pyrogen free water. The number of distillations has no advantage in freeing the water of the pyrogenic factor. One distillation is sufficient if the still is equipped with a spray trap. A minimum temperature of 140°C. is necessary for thirty minutes to completely destroy the specific substance. Pyrogenic substances require seven hours sterilization at 20 pounds pressure to completely destroy them. In media of pH 9.4 it requires five hours and a solution whose pH is 3.8 requires three hours at the same pressure in order to completely destroy it.

CONCLUSIONS

The reaction that follows intravenous administration of solutions is an entity characterized by an immediate and prompt rise of temperature in extremely high degree and usually associated with a severe rigor lasting about twenty to thirty minutes. This clinical picture is not to be associated with shock and is entirely separate and distinct from it. Intravenous reaction has a definite, specific, etiologic factor. This factor is the introduction of dead or living products of bacterial growth or cultures of—*Pseudomonas scissa* or *ureae*, into the blood stream of the individual.

These organisms were isolated in pure culture from the offending solution, reinjected into the individual and produced the same clinical picture. It was not possible to recover the organism from the blood stream of the individual suffering from a reaction, even though cultures were made at various times during the rigor.

The pyrogenic substance may be used as a therapeutic agent to produce hyperpyrexia.

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RETICULOCYTE COUNTS IN HEALTHY CHILDREN*

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The reticulocyte count has become firmly established as a valuable indicator of the state of bone marrow activity. It is useful not only as an aid in the diagnosis of anemias, but also as a guide to their effective treatment. It is surprising, therefore, that there is no readily available study of the reticulocyte counts of a sufficiently large series of healthy children of school age to serve as a basis for interpretation of reticulocyte counts in disease.

In the course of a series of studies of normal hematologic standards in adults^{1,2} and children* the optimum conditions for reticulocyte staining were investigated.³ This article is one of this series and reports the results of reticulocyte counts in children of school age by a method which showed more reticulocytes than the methods in common use.

SUBJECTS

Two hundred and eight children were studied. Of these, 106 were boys and 102 were girls. About ten children of each sex were included for each year between the ages of four and thirteen, inclusive. The age given was that at the last birthday. All had been found healthy at a recent physical examination. They include children from the grade schools, preschool clinic, orphanages, baby homes, and physicians' families. Many were children who were referred to the Doernbecher Hospital for tonsillectomy, but who, on physical examination, showed no indications for this operation. All social classes were included, but, since no significant differences were found in children from the different classes, the sources are not included in the table. All had leukocyte and

* Unpublished data.

differential cell counts and sedimentation rates which showed no striking deviations from the probability curve for normal values in a homogeneous group.* We believe them to be a thoroughly representative group of as nearly healthy children as it is possible to select.

METHOD

Reticulocyte counts were done by a technic which we have previously shown* gives optimum conditions for reticulocyte staining. The technic is briefly as follows:

Equal parts (5 drops) of well mixed oxalated blood and of 1 per cent brilliant cresyl blue in 0.85 per cent sodium chloride solution are mixed in a small test-tube. After one minute or longer a small drop of the mixed material is transferred to a clean slide and a thin smear is made in the usual manner. All reticulocytes in 1000 red cells from consecutive oil immersion fields are counted and the per cent computed. The figures reported in the table are the averages of two or more such counts of 1000 cells each.

Erythrocyte counts were done by the technic previously described.⁴ Apparatus with a Bureau of Standards certificate was used throughout. The results reported are the averages of counts made from two or more separate dilutions which agreed within 100,000 cells per cu. mm. of each other.

RESULTS

The results are indicated in table 1. The absolute values per cu. mm. were calculated from the erythrocyte count and the reticulocyte percentage. Note that there are no significant variations for reticulocyte counts at different ages or in the two sexes and that the average reticulocyte percentage of 1.47 for the entire group does not differ significantly from the average of 1.57 found in the study of 160 healthy adults.* The range of 0.4 to 3.8 per cent also corresponds well with the range of 0.5 to 3.8 per cent observed in adults. It is evident that the normal values for reticulocytes recommended for use with adults may also be used for children of this age group. These values are an average of 1.5 per cent and a range of 0.5 to 3.8 per cent.

The absolute counts average 71,473 per cu. mm. and range from 18,640 to 184,800 per cu. mm. They also show no significant variations with age or sex, but as one would expect from the

* Unpublished data.

TABLE 1
SUMMARY OF ERYTHROCYTE AND RETICULOCYTE COUNTS

AGE	SEX	NUMBER OF CASES	ERYTHROCYTES	RETICULOCYTES	
			million	per cent	per cu. mm.
4	M	11	4.80	1.44	69,188
	F	9	5.00	1.54	77,907
		20	4.89	1.49	73,067
5	M	12	4.93	1.38	66,442
	F	10	4.83	1.80	86,644
		22	4.89	1.57	75,645
6	M	8	5.00	1.64	81,080
	F	12	4.93	1.28	62,161
		20	4.96	1.42	69,728
7	M	11	4.92	1.23	60,305
	F	9	5.02	1.46	73,377
		20	4.96	1.33	66,188
8	M	16	5.16	1.23	62,109
	F	10	5.06	1.60	82,602
		26	5.12	1.37	69,991
9	M	14	5.10	1.40	70,967
	F	10	4.97	1.75	86,524
		24	5.04	1.55	77,449
10	M	8	5.11	1.44	72,976
	F	11	4.95	1.34	65,912
		19	5.02	1.38	68,886
11	M	9	5.24	1.32	69,633
	F	11	5.07	1.83	81,815
		20	5.15	1.50	76,333
12	M	10	5.21	1.47	77,431
	F	10	5.04	1.55	77,325
		20	5.13	1.51	77,378
13	M	7	5.29	1.14	60,404
	F	10	5.13	1.15	58,269
		17	5.20	1.15	59,148
All	M	106	5.02	1.36	68,541
	F	102	5.00	1.58	74,521
		208	5.01	1.47	71,473
Range	M		4.10-6.16	0.5-3.3	25,150-184,800
	F		4.05-5.86	0.4-3.8	16,640-184,650

erythrocyte counts they are lower than the corresponding values for men, 83,160 per cu. mm. with a range of 26,050 to 211,660, and similar to those for women, 72,800 per cu. mm. with a range of 21,400 to 156,800. This variation of the absolute values with the erythrocyte count, while the percentage count remains constant for each age and sex so far studied, is further evidence in favor of reporting reticulocyte counts in per cent rather than in absolute values. There is no apparent correlation between the erythrocyte count and the percentage of the reticulocytes.

COMMENT

These results average distinctly higher than results reported by workers using other methods. Since we have demonstrated that other methods fail to reveal all the reticulocytes actually present, a comparison of our results with such figures does not seem warranted.

It is noteworthy that with our technic no result below 0.4 per cent reticulocytes is found in either healthy adults or children. This permits one to conclude definitely that there is hypoplasia or aplasia of the bone marrow when few or no reticulocytes are found.

SUMMARY

1. Reticulocyte counts in 208 healthy children, about equally distributed as to sex and age between the ages of four and thirteen, inclusive, averaged 1.47 per cent or 71,473 per cu. mm. and ranged from 0.4 to 3.8 per cent or from 18,640 to 184,800 per cu. mm.

2. There were no significant variations in either the absolute or percentage counts for the different sex and age groups.

3. There were no significant variations in the percentage counts from the values previously found for men and women.

4. Reticulocyte counts should be reported in percentage rather than in absolute figures.

5. Normal values for reticulocyte percentage in adults or in children between the ages of four and thirteen, inclusive, are an average of 1.5 per cent with a range of 0.5 to 3.8 per cent, when a

method giving optimum conditions for reticulocyte staining is used.

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CARCINOIDS OF THE APPENDIX*

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Carcinoids of the appendix, because of their clinical behavior and histological structure, form a distinct tumor entity. Oberndorfer^{11, 12} was the first to distinguish this type of tumor from true cylindrical cell carcinomata. Although their apparent epithelial nature placed them in the latter category, nevertheless their failure to form local recurrences and to metastasize or produce a destructive growth, together with the fact that they occurred, as a rule, in the early decades of life, placed them in a separate group, to which Oberndorfer gave the name of carcinoid.

Prior to the present century, tumors of the appendix were rarely reported and only in the latter years has attention been focussed upon them. Norment,¹⁰ at the Mayo Clinic, found sixty-seven tumors in approximately 45,000 appendices. At Bellevue Hospital, no case of carcinoid of the appendix is recorded prior to 1916. Between January 1, 1916 and December 31, 1932, carcinoids of the appendix were encountered seven times amongst 9108 appendices. In the postmortem room at Bellevue, carcinoids of the appendix were found twice in 18,700 autopsies. It is probable that more painstaking technic would have revealed a few additional patients. The youngest patient in our series was twenty-three years old; the oldest fifty-four years; the average age was thirty-four and one-half years. Four out of seven patients were males. This is in contradistinction to the experience of most observers who report the lesion predominating in females. Clinically, in so far as a search of the literature reveals, a diagnosis of tumor of the appendix has never been made. In those cases where the lesion is not an accidental finding, a history of abdominal dis-

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comfort followed by signs of a perforated appendix or peritonitis is usually encountered. The classical symptoms of acute appendicitis, as described by Murphy, Deaver, and others are usually absent.

As to the pathogenesis of carcinoid of the appendix, two major and distinct theories have been advanced. One view, supported chiefly by Ribbert,¹³ Aschoff¹ and Oberndorfer, regards them as hematomas, or, as Aschoff designated them, naevus of the mucous membrane. On the other hand, Lubarsch,⁷ Miloslavich⁹ and others believed these tumors to be a type of true carcinoma arising from the epithelial crypts in the mucosa.

Subsequently, Gosset and Masson⁴ on the basis of histological examinations, believed these tumors to represent hyperplasia of special cells containing granules which are chromaffine in nature. Their histological appearance, similar to endocrine tumors, led them to believe they arise from endocrine cells, and hence called them "endocrine tumors of the appendix." Upon further investigation, using special silver stains, Masson⁸ noticed that these carcinoid cells were connected with fibers from the plexus of Meissner, and advanced the theory that these growths might be of neurogenic origin, admitting, though, that no proof to support this theory was available.

Danisch,² on the basis of painstaking histological work which included a study of the development of the structures comprising the appendix wall in the embryo, proved the theory of Masson and Gosset and showed that these chromaffine cells reduced ammoniacal silver and arose in the primary anlagen of the sympathetic ganglia from undifferentiated embryonal sympathetic elements, and that they can be found in the embryo as early as the fourth month. Using various stains, but especially the Bielschowsky-lithocarmine stain and Sudan III, hematoxylin-eosin, Danisch showed that these tumor cells, rich in lipoidal substance, which is at times noted even in the gross by the yellowish appearance of the tumor, represented a hyperplasia or possibly a regeneration following inflammation, of the argentaffine cells normally found in the intestinal canal (including the appendix) and originally described by Kultschitzky;⁶ later in greater detail by

Schmidt.¹⁴ Danisch believed that the elements comprising these tumors are related to the chromaffine tumors of the adrenal and, if so, expected to obtain an adrenalin-like substance from them. Because of lack of fresh material, he was unable to do this.

Hasegawa⁵ confirmed the work of Masson in its major part. Forbus,³ likewise using the silver stains, supported the theory advanced by these workers.

It is the object of this paper merely to confirm the presence of the silver reducing substance in the cells which form the so-called carcinoids of the appendix.

If these studies are correct, then the question arises as to the place of these tumors in oncology. They certainly are not true epithelial tumors; neither are they true neurogenic tumors. They are probably not carcinomata, neuroblastomata or sympathetico-blastomata. They do contain special pigment deposits and Danisch, acting on the suggestion of Pick and Bielschowsky, placed them in the group of pheochromocytomata.

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THE METHOD OF DILUTING ANTIGEN IN RELATION TO THE WASSERMANN REACTION

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Twenty-four years ago Sachs and Rondoni⁹ found that for the Wassermann test, alcoholic extracts of tissues diluted by adding salt solution slowly to the extracts with continuous shaking to secure turbid emulsions were more antigenic than when extracts and saline were rapidly mixed to produce opalescent or fairly clear emulsions. They also observed that turbid emulsions were slightly more anticomplementary than opalescent emulsions; that is, the former absorbed slightly more complement themselves than the latter.

In other words these investigators observed that turbid emulsions of alcoholic tissue extracts for the Wassermann test were more antigenic than opalescent emulsions but since then this important factor relating to the manner or method of diluting antigen for the Wassermann test has not received the attention that it deserves.

Browning and McKenzie² confirmed these observations and also noted that turbid emulsions of alcoholic lecithin-cholesterin extracts of ox liver were more antigenic than opalescent emulsions but that there were practically no differences in their anticomplementary properties when the complement had been kept for eighteen to twenty-four hours before use.

Noguchi⁶ in 1911 recommended the use of an opalescent emulsion of his extract of acetone-insoluble lipoids prepared by quickly mixing extract and salt solution as being "most certain in action" but in 1923⁷ advised adding the salt solution drop by drop to the extract to secure the maximum of turbidity and antigenic activity.

Kolmer⁴ advised the use of turbid emulsions secured by adding antigen slowly to saline solution and at this time Bronfenbrenner

and Schlesinger⁴ found that turbid emulsions of Noguchi's acetone insoluble lipoids were more anticomplementary but opalescent suspensions were more antigenic. Ruediger⁵ observed that the degree of turbidity greatly influenced the antigenic sensitiveness of extracts and that for each there seemed to be an optimum turbidity and an optimum dilution for maximum antigenic sensitiveness.

Kolmer and Trist⁵ in a study of this subject with various plain and cholesterolized alcoholic extracts arrived at the conclusion that the method of dilution had no apparent influence upon the hemolytic activity of extracts; that turbid emulsions secured by adding antigen slowly to saline or saline slowly to antigen were sometimes but not always slightly more anticomplementary than opalescent emulsions secured by rapid mixing but that *turbid emulsions secured by slowly adding extract to saline with constant shaking were more antigenic than opalescent emulsions secured by rapid mixing*. On the basis of these extensive comparative tests they recommended that antigen for the Kolmer modification of the Wassermann test be prepared by adding the antigen slowly to the saline with constant shaking to secure the maximum of turbidity and the maximum of antigenic sensitiveness.

More recently Wadsworth¹⁰ has advised the rapid mixing of cholesterolized extracts by placing the antigen in a liter flask and the saline in a second flask and pouring the saline on the antigen as rapidly as possible with thorough mixing back and forth several times.

Eagle⁷ however recommends a slow mixing to secure the maximum of turbidity by dropping two volumes of saline solution slowly and with shaking into one volume of his alcoholic extract of beef heart sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol and after two to five minutes adding 198 volumes of saline to give a 1:200 dilution. Such a dilution has been found more turbid and more sensitive than that prepared by dropping the antigen into the salt solution.

EXPERIMENTAL

This brief review of the literature shows that there is still some difference of opinion as to the best manner of diluting extracts for

the Wassermann test although it appears that the majority of investigators have confirmed the observations of Sachs and Rondoni that turbid emulsions are more sensitive or more antigenic than opalescent suspensions.

This certainly appears to be true of the Kolmer antigen (a cholesterolized and lecithinized alcoholic extract of beef heart) when turbid emulsions secured by slow mixing are compared with opalescent ones prepared by rapid mixing and both titrated at the same time by the Kolmer method. Sometimes there is but little or no difference between the two emulsions of the same extract but in the majority of instances the turbid emulsion, secured by adding antigen drop by drop to saline solution with constant shaking, has proved more antigenic than opalescent emulsions prepared by mixing antigen and saline very rapidly after the manner of diluting antigen for the Kahn precipitation test.

Since the Kolmer antigen is but rarely hemolytic even in a dose of 0.5 cc. of 1:4 dilution we have never been able to see any effect of the manner of dilution upon hemolytic activity.

Furthermore it would appear that there is but little or no apparent effect upon the anticomplementary activity of this antigen. In the Kolmer complement fixation method the guinea-pig blood is placed in the incubator for an hour or collected about twelve hours previously and kept in a refrigerator before the separation of the serum for complement.

With complement prepared in this manner turbid and opalescent emulsions of antigen gave almost identical anticomplementary units ranging from 0.5 cc. of 1:4 to 1:6 dilutions; but very occasionally the opalescent emulsions, secured by rapid mixing of antigen and saline, have been slightly more anticomplementary (0.5 cc. of 1:8) but never higher than 1:10.

It would appear therefore, that with this particular antigen the manner or method of dilution has practically no effect upon its anticomplementary properties.

There is usually a difference in the antigenic properties of this antigen according to the manner of dilution and in almost all instances the turbid emulsions have been more antigenic than the opalescent ones.

During the past ten years we have made comparative titrations

with twenty different Kolmer antigens. One emulsion of each was prepared by adding 1 cc. of antigen, drop by drop, with constant shaking to 3 cc. of saline to give a 1:4 suspension as recommended in the Kolmer modification of the Wassermann test; these emulsions were quite turbid and may be designated as "slow mixtures." At the same time 1 cc. of antigen was added very rapidly to 3 cc. of saline after the manner of diluting antigen

TABLE 1

ANTIGEN	SLOW MIXTURES (TURBID SUSPENSIONS)	RAPID MIXTURES (OPALESCENT SUSPENSIONS)
1	1:2400	1:1200
2	1:2400	1:1200
3	1:2500	1:2500
4	1:2000	1:2000
5	1:2500	1:2000
6	1:2000	1:2000
7	1:3200	1:2400
8	1:4800	1:4800
9	1:2400	1:2400
10	1:3200	1:4000
11	1:1600	1:2400
12	1:4800	1:2400
13	1:4000	1:3200
14	1:2000	1:2400
15	1:2400	1:2000
16	1:3000	1:2800
17	1:2000	1:2000
18	1:2000	1:1600
19	1:2400	1:2000
20	1:4000	1:4000
Average.....	1:2770	1:2465

for the Kahn precipitation test; these were opalescent and may be designated as "rapid mixtures." Further dilutions of each suspension were made with saline solution in the usual manner.

The antigenic units or the smallest amount giving + + + + reactions in titrations by the Kolmer method was 0.5 cc. of the dilutions listed in table 1.

With these twenty different C.J. extracts (Kolmer) the average antigenic unit of slow or turbid emulsions was 0.5 cc. of 1:2770

as compared with the average unit of 0.5 cc. of 1:2465 of rapid or opalescent suspensions showing the higher antigenic activity of the former. With seven of the extracts the antigenic units of the two suspensions were equal; with ten the turbid or slow mixtures were more antigenic while with three the opalescent or rapid mixtures were more antigenic. But the results have shown that in general terms the Kolmer C.L. extract possesses more antigenic activity when slowly added to saline solution to give turbid suspensions than when rapidly diluted with saline to yield opalescent suspensions.

TABLE 2

ANTIGEN	SLOW MIXTURES (TURBID SUSPENSIONS)	RAPID MIXTURES (OPALESCENT SUSPENSIONS)
1	1:6400	1:6400
2	1:3500	1:2500
3	1:2000	1:2500
4	1:8000	1:8000
5	1:6400	1:2000
6	1:6400	1:2400
7	1:3400	1:2400
8	1:4800	1:4200
9	1:2600	1:2600
10	1:2800	1:3000
11	1:2400	1:2400
12	1:4000	1:4000
Average.....	1:4350	1:3533

During the past two years comparative tests with twelve different Eagle antigens (alcoholic extracts of heart re-enforced with 0.8 per cent cholesterol and 0.6 per cent sitosterol) have shown similar results as shown in table 2.

Turbid emulsions may be also prepared by adding saline solution drop by drop with constant shaking to antigen. Indeed these are sometimes more turbid than when antigen is added drop by drop with shaking to saline solution.

Turbid emulsions prepared by adding 1 cc. of antigen drop by drop with constant shaking to 3 cc. of saline solution have usually proved more antigenic than turbid emulsions prepared by adding

3 cc. of saline in small amounts with constant shaking to 1 cc. of antigen. In one experiment with four Kolmer extracts the antigenic units are indicated in table 3.

Similar results were observed by Kolmer and Trist with plain and cholesterolized extracts tested with primary incubations of one hour in a water bath at 37°C. as well as eighteen hours in a refrigerator at 8°C. so that it would appear that the highest antigenic activity of an extract is obtained by adding the antigen drop by drop with constant shaking to the saline solution.

TABLE 3

ANTIGEN	ANTIGEN TO SALINE	SALINE TO ANTIGEN
1	0.5 cc. of 1:2600	0.5 cc. of 1:1700
2	0.5 cc. of 1:3000	0.5 cc. of 1:2000
3	0.5 cc. of 1:3200	0.5 cc. of 1:3000
4	0.5 cc. of 1:3000	0.5 cc. of 1:2400
Average.....	0.5 cc. of 1:2950	0.5 cc. of 1:2275

TABLE 4

ANTIGEN	BORDET MIXTURE	ANTIGEN TO SALINE	SALINE TO ANTIGEN
1	1:2400	1:2600	1:1700
2	1:2600	1:3000	1:2000
3	1:3000	1:3200	1:2400
4	1:2000	1:2400	1:2000
Average.....	1:2500	1:2800	1:2050

In conclusion brief reference may be made to the Bordet method of diluting antigen which consists of evaporating 1 cc. of extract on a watch crystal and adding 3 cc. of distilled water in amounts of 0.2 cc. while stirring with a glass rod after each addition, higher dilutions being made with saline solution in the usual manner. The resulting emulsions were but slightly turbid and less so than extracts slowly diluted by adding antigen to saline or saline to antigen, drop by drop, with constant shaking. Comparative tests with four Kolmer antigens gave the antigenic units in dose of 0.5 cc. as shown in table 4.

Bordet's method has been particularly recommended for the dilution of extracts of acetone-insoluble lipoids (Noguchi) but it is more troublesome, requires more time and is slightly inferior to the method of diluting by adding antigen drop by drop, with shaking, to saline solution.

CONCLUSIONS

(1) The manner or method of diluting extract for the Wassermann test has a slight but definite influence upon antigenic sensitiveness.

(2) Turbid emulsions of antigens secured by slow or gradual dilution with saline solution are more antigenic than opalescent emulsions prepared by rapid dilution.

(3) Turbid emulsions of antigens prepared by adding extract drop by drop to saline solution, with constant shaking, are more antigenic than turbid emulsions prepared by adding small amounts of saline solution to antigen with constant shaking.

(4) There is no detectable influence in the manner of diluting Kolmer antigen upon the hemolytic properties of this extract.

(5) Turbid emulsions of antigens prepared by slow dilution are sometimes slightly more anticomplementary than opalescent emulsions prepared by rapid dilution.

(6) In the Kolmer modification of the Wassermann test it is recommended that the antigen be diluted by adding it drop by drop to the required amount of saline solution with constant shaking to secure the maximum of turbidity as originally described in this method.

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THE CLINICAL AND PATHOLOGICAL FINDINGS FOLLOWING WARFARE GASSING*

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A study was made of the residual effects of gassing with chlorine, mustard, phosgene, and arsenical compounds, in the case of 486 veterans. Of this number 307 were living and 179 had died. One hundred and thirty-three patients had been gassed with chlorine; 142 with mustard; 139 with phosgene, and seventy-two had been gassed with arsenical compounds.

During the World War the various chemicals used for warfare gassing affected predominantly the respiratory tract. The character of the lesions found immediately after gassing depended upon the particular chemical substance used. Certain of these, as for instance chlorine, affected the upper portion of the respiratory tract; others, like phosgene and the organic arsenical compounds, affected the finer bronchi; mustard gassing was frequently accompanied by a secondary invasion of the respiratory tract with micro-organisms which resulted in acute suppurative conditions of the bronchi or alveoli.

Certain of the warfare chemicals caused traumatic injuries of the eyes. The severity and extent of the eye injuries were dependent upon the chemical used, as well as upon its concentration.

Mustard was the most effective of warfare chemicals in that it

* Abstract of a paper read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933. The study was conducted by Major General Harry L. Gilchrist, formerly Chief of The Chemical Warfare Service, U. S. Army and Dr. Philip B. Matz, Chief of the Research Subdivision, Medical and Hospital Service, Veterans' Administration.

The original studies appeared serially in the January, April, July and October, 1933, numbers of the Medical Bulletin of the Veterans' Administration, also in two brochures issued by the Chemical Warfare Service, U. S. Army.

caused the largest number of casualties. This study disclosed the fact that mustard gassing produced a large number of residual disabilities of various types.

It is believed that chronic bronchitis and emphysema were the most frequent and the predominant residual disabilities noted eight to ten years after gassing with most of the warfare chemicals.

In a number of instances quiescent or arrested pulmonary tuberculosis was reactivated due to the traumatization of the lungs by the warfare chemical.

Both the immediate and the residual effects of warfare gassing noted in this study were similar to acute or chronic infections with micro-organisms. The pathological and clinical findings following gassing were, in a great many instances, affected by complicating superimposed infections with various micro-organisms. This was particularly the case in subjects gassed with mustard.

Emphysema was found most frequently in combination with chronic bronchitis. In certain of the cases emphysema was diagnosed soon after gassing and in other instances it was noted as a residual condition. Emphysema in certain instances was compensatory and was due to extensive atelectasis or areas of congestion. In other instances it was due to an obstruction by an exudate or false membrane in the bronchi or bronchioli, which resulted in an impediment to the outgo of air from the pulmonary aveoli, thus bringing about a distention of the air sacs.

One of the outstanding pathological conditions noted after gassing, especially with phosgene and mustard, was the syndrome known as anoxemia. This condition was characterized by a shortage of oxygen and an increase of carbon dioxide in the blood, due to interference with normal respiration. This led to rapid and shallow breathing and tachycardia. If anoxemia was prolonged, myocardial degeneration was noted. Anoxemia was brought about by either a spasm of the bronchi or bronchioli due to toxic action of the warfare gas, or was the result of an exudate in the bronchi or bronchioli; in either case there was an interference with normal inflow of oxygen and outgo of carbon dioxide, thus an insufficient amount of oxygen was supplied to the tissues.

The effect of the various gases upon the heart was due to one or more of the following factors: (a) An absorption of the gas with resulting tachycardia; (b) a mechanical effect due to obstruction to the pulmonary circulation because of congestion or pulmonary edema, throwing an increased amount of work on the heart; (c) anoxemia resulting in an impoverishment of the blood supply and lessened oxygenation of the heart, with development of myocardial degeneration; (d) a complicating respiratory infection, such as lobar or bronchopneumonia, suppurative bronchitis or bronchiolitis, et cetera, with a deleterious effect upon the heart musculature.

Among the cases studied there were a number who gave a history of residual disabilities due to gassing, from which conditions they subsequently died. The most frequent causes of death among this group were active pulmonary tuberculosis and bronchopneumonia.

EDITORIAL

THE CLINICAL DIAGNOSIS OF BRUCELLIASIS IN MAN

With the advent of clinical appreciation that members of the genus *Brucella* are infective for man, the problem of clinical diagnosis soon became apparent on account of the varying pathogenicity of this group of bacteria.

Soon after the recognition of this infectious disease in America there arose a great deal of confusion when attempts were made to define what constituted a clear clinical picture. The inability of the bacteriologist to absolutely differentiate between the three more important members of *Brucella*, namely *melitensis*, *suis* and *abortus*, no doubt added to this confusion. As time elapsed and the clinical cases were carefully analyzed it soon became clear that *Brucella melitensis* was responsible for the undulating and recurring attacks of fever. *Brucella abortus* was of relatively low virulence often producing subclinical infections and seldom disease of long duration; the fever being of irregular character without the undulations.

During the first wave of enthusiastic interest every serum that agglutinated this group of *Brucella* was labeled an active infection, but since many of these individuals were symptom free and had questionable clinical histories, certain limits were placed upon the diagnostic significance of the agglutination reaction below 1:160 dilutions. This too was later proved to be of no diagnostic value since it was shown that proved cases of brucellosis with positive blood cultures had no agglutinins in their blood or agglutinated only in low dilutions. Serologic surveys revealed that serums from about 7 per cent of hospital patients agglutinated bacteria of the *Brucella* group and from healthy adults about 1 per cent. The agglutinin titers varied from 1:20 to 1:360. Thus it became apparent that the agglutination reaction had a limited diagnostic value.

The introduction of the intracutaneous reaction with *Brucella abortus* was also shown to have a limited value since the test behaves exactly like the tuberculin reaction in that it is only valuable in excluding the *Brucella* infection when the test is negative. Thus up to 1932 the only absolute evidence of Brucellosis was the demonstration of the infecting organism in blood cultures or by infecting guinea pigs with blood or pus from a suspected patient.

The recent introduction of the opsonophagic test by Huddleson et al.* offers a new possibility in establishing an absolute clinical diagnosis by laboratory methods without the demonstration of the organism by culture. Huddleson has shown that susceptible individuals have a low opsonophagic power while the immune have a high opsonophagic power. On the other hand the infected individual while exhibiting an opsonophagic reaction of less than the immune and more marked than the susceptible, can be easily differentiated from the susceptible individual by the aid of a positive intracutaneous reaction which is absent in the susceptible person. The writer's preliminary work with a limited number of experiments has so far substantiated Huddleson's report. It is hoped that clinical pathologists will undertake the investigation of this problem and will report individual experiences to the Research Committee for compilation.

—A. S. GIORDANO.

* HUDDLESON, F., JOHNSON, H. W., AND HAMANN, E. E.: A study of the Opsonocytaphagic power of the blood of allergic skin reaction in *Brucella* infection and immunity in man. Am. Jour. Pub. Health, 23: 917-929. 1933.

NEWS AND NOTICES

THE THIRTEENTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS AT CLEVELAND, JUNE 8 TO 10, 1934

The programs of the Society have in the past been so superior that it would seem impossible to reach the high standard set at previous meetings. Nevertheless, that it can be done is clearly seen by the tentative program listed below. The Thirteenth Convention promises to be in every way up to standard with any of the past programs. Members who will be forced to miss the meeting will miss not only a profitable but a very enjoyable experience.

The Executive Committee will meet on Thursday evening. There will be a round-table discussion on Friday evening conducted by Dr. P. F. Morse of Detroit following a supper at the Hotel Cleveland. It will deal with the Clinico-Pathologic Conference as well as other interesting questions. The Annual Banquet will be held at 7:30 P.M. Saturday evening. The principal speaker will be Dr. Howard T. Karsner, of Western Reserve University. The business meeting will be held on Sunday evening; the local committee is planning a supper and the meeting to follow immediately after. In general, the program this year will emphasize the subject of malignancy as well as the very pertinent subject of hematology.

Below is a tentative program which, although it will probably be necessary to make certain changes in it, is indicative of the excellent quality of the material which will be presented.

TENTATIVE PROGRAM

Friday, June 8, 1934, 9:00 A.M.

Short Business Session

SCIENTIFIC PROGRAM

Erythroblastic anemias of childhood. A. Yaguda.

Etiology of granulopenia (agranulocytosis)—with particular reference to drugs containing the benzene ring. R. R. Kracke and Francis P. Parker.

- Treatment of malignant neutropenia by injection of liver extract. W. B. Martin.
- Subleukemic states and leukemoid reactions. A. S. Rubnitz.
- Morphologic data in the leukemias. F. J. Heck.
- Heterophile antibody reaction in infectious mononucleosis. N. Rosenthal and George Wenckebach.
- Blood studies in animals following peptone injection. Harry Goldblatt and E. A. Greenspon.
- Direct color photomicrographs by the Finley process on all types of blood cells and abnormal bloods. Russell Haden.
- Medico-legal application of isoagglutinins. H. A. Heise.
- Further studies on antigen emulsion preparation for the ball test for syphilis. B. S. Kline.
- Results obtained with a uniform, stable and lyophile complement. Harry Eagle, Henry Strauss and Rudolph Steiner.

Friday, June 8, 2:00 P.M.

- The mechanism of jaundice. N. Elton.
- Morphogeographic studies of the thyroid gland, obtained at autopsy. C. A. Hellwig.
- Report of an unusual case of siamese twin monstrosity. L. W. Larson.
- Oil aspiration pneumonia. Kano Ikeda.
- Pyrogen produced hyperpyrexia in treatment of central nervous system lues. H. M. Banks.
- Paper on neuropathology. J. W. Kernohan.
- Pathological changes resulting from hypo- and hyper-secretion of ductless glands. Anna May Young.
- A postmortem analysis of 1000 cases of peritonitis. C. C. Pflaum.
- Adrenal diseases in relationship to hypoglycemia and death. J. C. Norris.
- Cyanide poisoning: An analysis of cases occurring in New York City since January 1, 1918. A. O. Gettler, and A. V. St. George.

Saturday, June 9, 9:00 A.M.

- Paralytic accidents due to anti-rabic inoculations. F. C. Hodges.
- Significance of presence of fungi in various locations in the body. Stephen H. Curtis.
- Subacute bacterial endocarditis due to diphtheroid bacilli. A. G. Foord and W. J. Stone.
- Clinical significance of non-glucose sugars in the blood. R. J. Pickard.
- Dextrose tolerance test. W. G. Exton and Anton R. Rose.
- Determination of arsenic. A. E. Osterberg.
- Diagnostic methods in amebiasis: relative value of stool culture as compared with other methods. C. J. Tripoli and Morris Shushan.

Saturday, June 9, 2:00 P.M.

Early diagnosis of cancer of the stomach. Wm. Carpenter MacCarty.
Malignant hemangioma. B. Markowitz.
Pathogenesis of lung cancer. H. C. Sweany.
Primary carcinoma of the lung. O. A. Brines.
Clinical—pathological aspect of intestinal fibromatosis. Leo F. Bleyer.
Grading of cancer for prognosis. B. Steinberg.
Precancerous lesions of the breast. Max Cutler.
An attempt to define a neoplasm by way of the processes of proliferation, differentiation, and organization. S. P. Reimann.

REGISTRY OF TECHNICIANS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The first nation wide examination for applicants for certification by the Registry was held last October and proved a pronounced success. The questions were eminently fair judging from the comments of both the examiners as well as those seeking recognition by the Board.

The test took the form of a practical as well as a written examination, the questions having been formulated by a member of the Board of Registry and the examination conducted by a Fellow of the Society or a recognized clinical pathologist in the town nearest the residence of the applicant.

The examiners cheerfully gave of their time and facilities in conducting these tests and conscientiously carried out their duties. The first examination brought sixty-six applicants of whom fifty-six successfully passed. Thirty-two examiners participated in the event.

The second examination was held late in April, the results of which are not yet available. Over one hundred and twenty-five aspirants from the United States and Canada filed applications for this examination.

The Board of Registry will hold its annual meeting in Cleveland during the convention of the American Society of Clinical Pathologists. Among the matters to be taken up will be the award of the title of Medical Technologist to those who deserve this designation. Sessions will be devoted to the formulation of a curriculum for training schools and in general to the elevation of the scientific status of the clinical laboratory technician.

Members are urgently requested to attend, and all registered technicians are invited. Non-members will be accorded all privileges except voting. Business sessions are scheduled for Monday, June 11, at 10 a.m., and Wednesday, June 13, at 9 a.m. Scientific meetings will be held Monday afternoon and Tuesday morning. Tuesday afternoon is open for hospital tours, and the annual dinner will be Tuesday evening.

Speakers for the meetings will be:

Dr. Philip Hillkowitz, Chairman, Board of Registry, Denver, Colorado. "The Relation of the Registry of Technicians to the American Society of Clinical Laboratory Technicians."

Dr. B. S. Kline, Mt. Sinai Hospital, Cleveland, Ohio. "The Kline Test for Syphilis."

Dr. Asher Yaguda, Beth Israel Hospital, Newark, N. J. "The Requirements and a Tentative Curriculum of Approved Schools for Technicians."

Dr. W. D. Stovall, Director of State Laboratories, Wisconsin Board of Health. "Molds and Fungi and How They Effect the Technician."

There will also be scientific papers read by members of the society.

The Convention headquarters will be the Carter Hotel, Prospect Avenue and East Ninth Street, Cleveland, Ohio. Send reservations for annual dinner to Mrs. Christine C. Seguin, 8056 Lincoln Avenue, Niles Center, Ill. Tickets for the dinner will be sold in Cleveland for \$2.00.

Word has reached the Registry of the formation of the Texas Society of Clinical Laboratory Technicians. An enthusiastic meeting was held in San Antonio, December 9, 1933. Constitution and By-Laws were adopted. Committees were appointed and the following officers elected:

President, Mr. H. A. Bardwell, San Antonio.

First Vice President, Mrs. Pauline S. Dimmitt, Sherman.

Second Vice President, Miss Elizabeth Pickett, Dallas.

Secretary, Mrs. Ida F. Levinson, Houston.

Treasurer, Mr. George T. Thomas, Beaumont.

At the banquet, the following physicians addressed the meeting: Dr. T. S. Roach, Dr. B. F. Stout and Dr. J. H. Moore.

The next meeting will be held in Dallas, Texas, during the week of the State Fair.

BOOK REVIEWS

Aids to Pathological Technique. By DAVID H. HALER. Pp. x + 187. Baltimore, William Wood and Co., 1933. \$1.50.

This pocket-sized manual is intended for students and other laboratory workers and especially for those about to take examinations. The author has selected only one method for each test and has avoided any but the briefest discussions. An idea of the content of the manual may be gained from the section headings: Bacteriology, Hematology, Cytology and Parasitology, Biochemical Methods, Preparation of Media et cetera and Stains and other Formulas. The methods given frequently are those known in England but not used in the United States and the fact that inoculation of guinea pigs for tuberculosis is omitted because they are done only in laboratories licensed by the "Home Office" will not help the rest of the world. A large number of colloquial expressions and references to old world apparatus appear. The nomenclature in Bacteriology is badly mixed with old and new names. Some examples of careless writing occur, as for example, reference to cultural methods for "Cysticereus hooklets of various worms;" abbreviations in a table on page forty-three are not apparent; Taenias are considered as a class on page ninety-four, as separate from other cestodes, and tapeworm infestations are supposed to be diagnosed by finding the worm or its scolex, no mention being made of the ova. The advice to draw blood from the ear by puncturing with a glass capillary is certainly dangerous and uncalled for. The omission of flocculation tests for syphilis because the Ministry of Health requires the Wassermann well indicates the breadth of the book.

Text-Book of Pathology. By ROBERT MUIR. 3rd Ed. Pp. vii + 957. Baltimore, William Wood and Co., 1933. \$10.00.

This edition contains many important changes in the text made necessary by the advance in knowledge of the subject and some

parts have been rewritten. A number of additional illustrations to the already numerous ones have been included. They are all of excellent quality and most of the photographs are from specimens in the Museum of the Western Infirmary, Glasgow. While the presentation of the subject is from the standpoint of a teacher of pathology and hence is intended as a text, the author emphasizes the relation to clinical medicine and surgery. The book is divided along orthodox lines and deals with general and special pathology. While the text is clearly written and the presentation of the subject good, a distinct criticism is the lack of references to original articles. The student should be encouraged to read outside of the text by giving him numerous references. For example, if this had been done he might discover that the oncosphere of *Diphyllbothrium latum* has a very definite way of reaching a fresh water fish and does not get there "in some way" as indicated on page 454. Also it was Gosset and Masson who described the silver reaction in carcinoids of the appendix and who spoke of "endocrine adenomas" and not Masson alone. Masson, eight years later put forward the theory of the origin of certain cells in these tumors from nervous tissue, a fact omitted in the text. The book contains an elaborate index.

Histology. BY S. RAMÓN-CAJAL, REVISED BY J. F. TELLO-MÚÑOZ, TRANSLATED FROM THE TENTH SPANISH EDITION BY M. FERNÁN-NÚÑEZ. Pp. xiv + 738. Baltimore, William Wood and Co., 1933. \$8.00.

American students will receive with pleasure this English edition of the most famous Spanish text in anatomy from the master histologist of the present time. Because Cajal's major works have been published in his native tongue most American students have not known of his investigations at first hand. This text is especially complete and beautifully illustrated with 535 figures, most of which are original or from the author's pupils. The first chapters are concerned with cytology and early embryology. Then follow chapters dealing with the tissue systems. These are followed by consideration of the histology of the organ systems, and finally by chapters dealing with methods which are especially

valuable. Naturally the outstanding chapters deal with the nervous system and here one finds a treatise of about 200 pages and in addition twenty pages on neurological technic. These chapters give a complete record of the great master's contributions to the subject and make available quickly, material otherwise almost impossible to obtain. References throughout the book are limited to Spanish investigations. The book is as thorough and comprehensive as a text should be and may be accepted as entirely authoritative.

THE RÔLE OF THE PATHOLOGIST IN THE CANCER PROBLEM*

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In 1931, Dr. C. C. Little in one of the International Contributions to the volume of the Annals of Surgery dedicated to James Ewing, made the statement that there are four great fields of work in which new advances are necessary in the control of cancer:

(1) More satisfactory and complete methods of classifying and of differentiating between the various types and degrees of cancerous growth will have to be established.

(2) Best possible methods of treatment must constantly be subjected to close scrutiny with the view in mind of improvement and refinement and of the development of new lines of attack.

(3) The immensely important work of attempting to decrease the incidence of cancer by education of laymen must be carried on.

(4) Research as to the cause or causes of uncontrolled cancer growths must be fostered.

He further wrote:

In all these problems the need of larger numbers of better trained medical men is paramount. Because of many reasons, among which the difficult and discouraging nature of cancer itself is undoubtedly one, the medical schools and active medical profession give much too little attention to training would-be or young doctors in the problem of cancer. Instead of recognizing the challenge offered by cancer as being an outstanding menace about which pathetically little is known and for the combating of which the utmost coöperation is needed, the medical profession fights shy of grappling with the problem and looks for easier foes to conquer. This attitude will have to be corrected. The spread of popular interest in cancer, coupled with a marked decrease in ignorant fear and superstition concerning it, will undoubtedly in the near future result in a

*Presidential Address read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

popular demand by the laity for better equipped medical men to cope with the situation. Then, if not before, medical schools and the medical profession as a whole will take the long overdue steps toward more up to date and extensive training of their personnel.

In the last few years an active response has been made to this challenge, and the fight against this disease has been waged with a vigor not known in the past, and throughout most States a sense of cancer-consciousness is being instilled into the medical profession. Most pathologists have fallen into line in this great battle and are endeavoring to aid in the first three of the methods of attack laid down by Dr. Little, and a few are able to carry on research on the cause of cancer. Some pathologists, however, have failed to realize their key position and have been made the object of attack by various critics.

I am not in accord nor do I believe this audience agrees with a statement in a recent editorial in the January, 1934, issue of the American Journal of Cancer, which ends with this paragraph:

What medicine needs today is, first, personality, and second, wide training, both in the laboratory and the clinical aspects of the art. The lack is more often in the laboratory phase.

Personality certainly plays its part, but I feel certain that the status of pathological diagnosis of cancer is at present far ahead of the clinical aspect of diagnosis, and far superior to the average treatment afforded the cancer patient. That poor pathological diagnoses are made in some places in this country, none can deny, but by using the well-trained pathologists now available in the manner suggested by this Society and the American College of Surgeons, namely, by having a pathologist serve more than one hospital in smaller cities, conditions would be improved immensely and the untrained men would have to brush up or quit. This method would not correct the entire situation at once, but if profitable financial futures are guaranteed young men by hospitals and support by their confreres in clinical medicine, an increased supply of better trained men will be furnished to those places where they are needed. I believe that organizations endeavoring to strengthen scientific medicine and particularly labo-

ratory diagnosis should exert their major efforts on delinquent hospitals and lukewarm clinicians so they might arrange for satisfactory financial support of good pathologists. This should be done rather than endeavoring to change the entire scheme of the practice of pathology by advocating that it be practiced by men whose plan is to use the position as pathologist as a mere stepping stone toward clinical medicine or surgery.

Those already in the field have abundant opportunities to aid in the cancer campaign and can serve as others cannot on our hospital staffs. Our position is the corner stone of the problem in the individual patient and not merely that of diagnosing the type of tumor under a microscope, perhaps several days after a surgical specimen is sent to a laboratory. It should begin in many cases before clinical diagnosis is made or treatment started. We should be, as Dr. Simpson stated last year in his presidential address, consultants, paid or otherwise, and if our clinical training has been slighted in the past, tumor cases will certainly furnish material for increasing our own diagnostic abilities. Following this we should be on hand in the operating room to give opinions on gross tissue specimens followed by frozen sections if necessary, in order that proper treatment may be established immediately. It has been my experience that the better surgeons are anxious for all the detailed information they can get. Also, more patients every year are asking for prompt diagnosis on their tumors before mutilating surgery is done. We must furnish this service to accomplish properly our work.

I should not need to mention to a group of pathologists that in case of death a necropsy should be performed on all cancer patients, but a look at the post mortem statistics in many hospitals will convince any one that many are missed. It is common experience that post mortem consent is easier to obtain in cases dying from cancer than from other causes of death, and often a little gentle prodding from the pathologist will induce an attending physician or his assistant to gain the necessary permission. Particularly important are post mortem studies of cancer patients treated by roentgen ray or radium, especially in those given the stepped up dosages used in well equipped clinics at the present

time. Without such studies we can never know what is being done by these agents. Incidentally it is important that the pathologist acquaint himself with the picture presented by heavily radiated tissues otherwise he may make pitiful mistakes in cases of biopsies or surgical specimens from treated cases.

Furthermore, the pathologist should be one of the most active individuals in the cancer clinic in his hospital, or, if none exists, he should endeavor to impress on his staff the necessity for such a clinic. He, having no axe to grind and being in a neutral position, can often serve as the arbitrator in disputes between the radical surgeon and the over-enthusiastic radiotherapist, or between the over-pessimistic internist and the zealous dermatologist. With a little extra work on his part he can demonstrate gross material and microscopic preparations of tissues removed from patients before or after presentation before the clinic. Most clinicians are anxious to see such material if presented properly.

In smaller hospitals particularly, the pathologist can aid a great deal in teaching his own staff by summarizing and analyzing the records and end results in cases of malignancy treated in his own hospital, where such attempts by one or more clinicians might not be favored by some of the staff because of professional rivalry, personal or other reasons. By such analysis the results of various methods of treatment can be evaluated, mistakes in diagnosis and therapy can be impersonally discussed, and pertinent data can be obtained as to the variation in the clinical course depending on the type and grade of the tumors, the location and extent of the involvement, et cetera. This material may not be as abundant as in some larger institutions, but oftentimes it is more interesting, since the cases are those actually seen by the local men, including not only the man who treats only an occasional case but also those who handle larger numbers but never stop long enough to take stock of their final results.

Also the pathologist, realizing the necessity for more information about cancer among clinicians and pathologists alike, should see to it that case reports or papers on malignant disease be presented more often on the programs of staff meetings or those of local, county, state, or even national organizations. He

should take the time and effort to work up interesting cases, including gross and microscopic photography for presentation by the clinician or himself, and although it may cost him a large amount of labor and effort, in the long run the knowledge gained will be worth the price, and the cementing of friendship with his clinical confreres will more than repay him.

Before closing, may I mention a few methods which we as a Society should support or sponsor in disseminating information on the pathological diagnosis of tumors to pathologists. Needless to say, the best way is to take time off and study at some of the larger clinics here or abroad, but in these days this cannot be done except by a few. Failing that, we can bring the tissues to the pathologist. This we have started in the form of our Tumor Registry under Dr. Brines' committee who are collecting slides accompanied by histories, et cetera of tumors from all organs of the body. These will be sent out as loan slides (five or more sets will be available) and should serve as topics of discussion for small groups of men. This registry is in no way to conflict with the Bone Tumor Registry of the American College of Surgeons or the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists. On the contrary, support of these registries is more than desired since material sent to them serves as a source of information in the reports of the study of the large numbers submitted, and also the individual pathologist who sends in a case has the benefit of the best consultation in the country.

Furthermore, our state counsellors or men chosen by them should organize small units of pathologists in places where no regular pathology society exists, as has been done in some of the states already. By a little effort arrangements can be made for visits by men of larger experience, and in turn the visitor, I am sure, will be repaid by seeing material fully as interesting as in his own hospital or university.

Another scheme which works splendidly and which might serve as a model for other states is the holding of semiannual round table meetings as is done under the auspices of the Cancer Commission of the State Medical Society in California. Eight or

ten cases are presented clinically, gross specimens or lantern slides are demonstrated, and then the slides of the tissue are examined, each man having his own slide, followed by a vote on the diagnosis. The man presenting the case then discusses his own diagnosis, followed by general discussion. An average of thirty-five to forty pathologists attend the meetings and return with a full set of slides of rather difficult tumors for future reference. These meetings, if they serve no other purpose, serve as a stimulus to those men who have poorly made slides to pay attention to this very vital point in tissue diagnosis.

Finally, in behalf of the tumor patients of this country who come to medical men, may I recommend that we all consider the subject of tumors more fully than ever, not only from the standpoint of how many mitotic figures there are in a microscopic field but from the viewpoint of curing the particular patient we are interested in, and from the general viewpoint of the handling of the entire cancer problem. We have as yet only scratched the surface. Let us dig deeper, help when we can, and above all, furnish accurate information and proper diagnoses from our own laboratories, scrutinize carefully new developments of treatment, stress cancer to our students and keep our colleagues alive to the subject.

CLINICO-PATHOLOGICAL RELATIONSHIP IN COMMON BREAST LESIONS*

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The statistical portion of this survey is based on the clinical and laboratory records of 255 patients admitted to St. Luke's Hospital, Davenport, Iowa, and St. Anthony's Hospital, Rock Island, Illinois, for the diagnosis and treatment of breast disease, within the ten year period from 1924 and 1933, inclusive. During this decade the total combined admissions were 34,464, exclusive of dispensary and out patients, but including an undetermined number of readmissions.

Since the average number of patients in each institution has been approximately the same, the first five of the following tables are arranged so that the statistical data in both series may be compared. In the remaining two tables, the totals are combined.

From table 1, it will be seen that nine patients were considered to have inoperable malignancies, and that thirteen, presumably with carcinomas, were treated nonsurgically, although there was no histological verification, and both groups have been eliminated from further consideration in this report. In 214 cases from which histological examinations were made, no pathological lesion was recognized in nine instances, other than simple or involutional atrophy.

As indicated in table 2, the incidence of malignant disease comprised about one-third of the total number; benign tumors, a little less than one-half; cystic disease, approximately 11 per cent, and inflammatory lesions 6 per cent.

In this series, the malignancies were not classified originally with a view to grading the degree of anaplasia, except in so far

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as the general histological architecture of the neoplasm might serve that purpose. The terms adenocarcinoma, scirrhus and medullary carcinoma have long been used to designate respectively the tendency to gland formation, a relative abundance of

TABLE 1

	ST. LUKES	ST. ANTHONY	TOTAL
Breast cases (1924-1933).....	137	118	255
Incised and drained.....	2	5	7
Inoperable (carcinoma).....	4	5	9
Treated nonsurgically.....	1	12	13
Examined histologically.....	121	93	214
Acquired anomalies (adult hypertrophy).....	3	0	3
No lesions recognized*.....	5	4	9

* Except simple or involutinal atrophy.

TABLE 2
INCIDENCE OF VARIOUS LESIONS

LESIONS	ST. LUKES	ST. ANTHONY	TOTAL	
			Number	Per cent
Malignancies.....	42	31	73	35.6
Benign tumors.....	48	42	90	43.9
Cystic disease.....	14	9	23	11.2
Inflammatory lesions:				
Acute pyogenic (multiple abscesses	5	7	12	5.8
Chronic mastitis (exudative and fibrous)				
Tuberculosis.....	1	0	1	0.5
Anomalies:				
Gynecomastia.....	2	0	2	1.0
Adult hypertrophy.....	3	0	3	1.5
Unclassified.....	1	0	1	0.5
Totals with recognized lesions	116	89	205	

fibrous connective tissue stroma, and solid masses of cancer cells with but little fibrous stroma. Deductions drawn from early studies in histological prognosis implied that adenocarcinomas were the least malignant, scirrhus types occupied an intermediate position, and medullary carcinomas were the most malig-

nant.² Follow-up statistics have not always borne out these implications, particularly with reference to differences between the scirrhous and medullary types. In reviewing my descriptions of histologic sections in individual cases, I find that I have referred frequently to the number of mitotic nuclear figures, to the chromatic character of the nuclei, pleomorphism, and variation in size of both cells and nuclei, to the diffuse or circumscribable character of carcinomatous infiltration, to the presence of inflammatory or lymphocytic infiltration, and to evidence of secretory activity of both parenchyma and stroma. These observations

TABLE 3
MALIGNANCIES

TYPE OF TUMOR	ST. LUKES	ST. ANTHONY	TOTAL
Scirrhous.....	17	10	27
With extensive fibrosis.....	2	0	2
Far advanced.....	2	1	3
Adenocarcinoma.....	3	4	7
Papillary cyst.....	0	3	3
Partly scirrhous, alveolar, or medullary.....	8	5	13
Medullary.....	7	5	12
Paget's disease of nipple.....	1	3	4
Sarcoma:			
Alveolar.....	1	0	1
Fibrosarcoma.....	1	0	1

were made in a purely objective manner, however, and without regard to any formula or formal system for histological prognosis. A correlation of these and other factors has been undertaken by the reëxamination of sections from the paraffin blocks in this series combined with a larger group of breast cases. There is at present, a wide difference of opinion among pathologists regarding the merits of histological grading.

Ninety cases (44 per cent) of the 205 cases in which pathological changes were recognized were classified as benign tumors. Solid adenofibromas made up about 60 per cent of the group of benign tumors, while cystadenofibromas and adenofibromas with widely dilated ducts accounted for approximately 25 per cent.

As a practical point, cystadenomas which contain blood, those lined by soft partly necrotic tissue, and those which have thick rigid walls should be carefully examined for evidences of malignant transition. Early carcinomatous change was observed in one cystadenoma in this series.

TABLE 4
BENIGN TUMORS

TYPE OF TUMOR	ST. LUKES	ST. ANTHONY	TOTAL
Adenofibroma:			
Peri- or intracanalicular.....	21	28	49
With myxomatous degeneration.....	2	0	2
With diffuse mastitis.....	3	1	4
Cystadenofibroma.....	12	8	20
With early malignant change.....	1	0	1
With chronic mastitis.....	1	0	1
Fetatic ducts only.....	2	0	2
Adenoma.....	3	2	5
Lipoma.....	3	3	6

TABLE 5
CYSTIC DISEASE AND INFLAMMATORY CHANGES

LESION	ST. LUKES	ST. ANTHONY	TOTAL
Cystic disease:			
Large and small cysts.....	14	9	23
Adenomatous hypertrophy of lobules.....			
Galactocoele.....	1	0	1
Inflammatory changes:			
Acute, pyogenic (multiple abscesses).....	1	5	6
Chronic mastitis (exudative and fibrous).....	3	2	5
Tuberculosis.....	1	0	1

Twenty-four cases (12 per cent) of the total were regarded as cystic disease of the breast (Reclus's disease). In certain stages of development the distinctions between retention cysts, cystic disease, or cystic mastitis, and cystadenomas are not always clear cut. Usually the character of the stroma, and the absence of small satellite cysts help to differentiate cystadenomas from cystic mastitis. Retention cysts often contain a liquid or semisolid

milky fluid. That type of cystic disease in which there are solid masses of cells presenting microscopically an adenomatous hypertrophy of the lobules is of special interest since it may be mistaken for adenocarcinoma. This has been considered by some pathologists to be a precancerous lesion, yet it differs sharply from carcinoma in that the glandular hyperplasia retains a lobular arrangement. So far as can be determined from follow-up records in this small series there has been no instance of carcinoma developing after simple excision or mastectomy for cystic disease.

In table 6 an attempt has been made to correlate the incidence of palpable lymph nodes as reported in the clinical histories with the post-operative examination of tissues, and the mobility or

TABLE 6
ADENOPATHY AND MOBILITY

	GLANDS—PRE- OPERATIVE EXAMINATION			GLANDS—POST- OPERATIVE EXAMINATION			MOBILITY OF TUMOR		
	Pres- ent	Ab- sent	Unre- ported	Pres- ent	Ab- sent	Unre- ported	Fixed	Free	Unre- ported
72 malignancies.....	38	25	19	47	21	4	33	29	10
80 benign tumors.....	10	48	12	4	62	14	3	64	13
20 cystic disease.....	2	15	3	1	14	5	2	17	1

fixation of the various breast lesions as recorded in the clinical findings. A number of clinical records in the series had to be discarded due to incomplete data. Not all of the records from which table 6 is made up were as complete in this respect as might be desired; the deficiencies are indicated in the table in columns headed "unreported." For example, in the seventy-two malignancies, palpable lymph nodes were noted in the clinical histories in thirty-eight cases; absence of palpable nodes was noted in twenty-five cases; and no notation was made in nine cases. In the gross examination of the tissues, enlarged lymph nodes were found in forty-seven cases, no nodes were found in twenty-one, and in four instances notation was not made. The figures set down opposite the eighty benign tumors, and twenty cases of cystic disease are to be interpreted in the same

manner. Summarizing, in a general way, that portion of the table referring to adenopathy, it will be noted that palpable lymph nodes were found preoperatively at the time of examination in about one-half of the patients suffering from malignancies. Post-operatively enlarged nodes were found in approximately two-thirds of these cases. Enlarged lymph nodes were found rarely in association with the benign tumors and cases of cystic disease, although other palpable masses were occasionally mistaken for enlarged nodes.

Mobility, as the term is used in this table, refers not alone to nodular fixation or freedom of movement, but in order to simplify the analysis, it includes dimpling of the skin, retraction of the nipple and subdermal thickening. Only those breast nodules were classified as freely movable which were not fixed, and which were unaccompanied by retraction, dimpling and subdermal thickening. In twenty-nine cases (40 per cent) of the malignancies of this series, the neoplasm was freely movable at the time of examination. It should be remembered that malignant nodules centrally located within the breast remain movable until extension and infiltration involve the dermal or subdermal lymphatic spaces, or the underlying muscle fascia. It is an important point that these well known clinical signs are of diagnostic value only when they are present; their absence should not be taken as evidence against malignancy, especially in the case of an intramammary nodule.

There is possibly no better illustration of the advantage of coöperation between surgeon and pathologist, than in the management of breast disease. That coöperation is especially desirable in the most common manifestation of breast disease, namely the solitary mobile nodule. By means of the frozen sections, the character of its histologic structure may be determined within a few minutes, and the scope of the operation planned accordingly. Experience with frozen sections over a period of years has repeatedly demonstrated their practical worth. Moreover, exploratory operations should not be performed unless the surgeon is prepared either alone or with the aid of a pathologist to establish the diagnosis and proceed with the radical operation,

if carcinoma is found. The less experienced surgeon, who relies on his knowledge of gross pathology alone assumes an unenviable degree of responsibility.

From the standpoint of age incidence, about 85 per cent of the benign tumors occurred in patients under fifty years, while approximately the same percentage of malignancies were found in persons over forty years of age. The age incidence of cystic disease approximated that of benign tumors.

TABLE 7
AGE GROUPS IN THE COMBINED SERIES

AGES	MALIGNANCIES	BENIGN TUMORS	CYSTIC DISEASE
10-19	0	1	0
20-29	1	15	4
30-39	6	36	5
40-49	23	32	8
50-59	18	3	3
60-69	14	4	1
70-79	9	1	0
80-89	2	1	0

DIFFERENTIAL DIAGNOSIS

It is important to determine promptly and decisively the nature of breast nodules. There are but few, if any, valid reasons for such advice as "to wait and see what happens." It would be far safer for the patient if the physician were to assume that all breast nodules were malignant until proved otherwise.

Errors in diagnosis, and consequently the treatment based on that diagnosis if uncorrected, occur most commonly under three headings:

(1) A small, circumscribed, freely movable, isolated nodule without any sign of attachment to the skin or nipple occurring in a young woman often leads to the diagnosis of adenofibroma, when actually the nodule is malignant. As pointed out in the discussion under table 6, nearly 40 per cent of the malignant nodules in this series were movable and without skin attachment; and as shown in table 7, 12 per cent of the breast lesions in women under forty years of age were malignant.

(2) A slowly growing, firm, circumscribed mass unattached to the skin or underlying muscle, and without palpable axillary glands is assumed at times to be benign, on the basis of a long history. Actually such a nodule may well be a duct carcinoma, or slowly growing cystadenocarcinoma free of attachment in spite of its size.

(3) The presence in one or both breasts of more than one tumor throws the weight of evidence against malignancy, and favors the diagnosis of benign lesions.¹ Multiple tumors may be fibroadenomas, or still more likely some form of cystic disease. Pain and tenderness are less frequently associated with fibroadenomas than with cystic disease. A conservative operation may be planned therefore in the treatment of multiple painless nodules, and if at operation these tumors are found to be solid, local excision with a margin of normal tissue is adequate. On the contrary, mastectomy is the operation of choice in multiple cystic tumors. Both large and small cystic nodules are frequently surrounded by an area of smaller microscopic cysts extending for some distance into the mammary tissue. While the larger cysts are not likely to undergo malignant transition due to degeneration of the glandular epithelium, the epithelial cells of the smaller cysts are more active and probably more responsive to whatever stimulus induces malignant change (Schimmelbush's disease). Although malignant transition is uncommon, to remove the larger cysts and leave the smaller ones is to remove the more innocent lesion and leave behind the more dangerous one.¹

SUMMARY

A knowledge of the clinico-pathological relationships in breast disease is the basis for its intelligent management. The simple classification of benign and malignant tumors, cystic and inflammatory disease covers the vast majority of lesions with which one may be confronted. Some of the natural limitations in the differential diagnosis of the most common breast lesion, namely the solitary mobile nodule, have been emphasized in a statistical way. It has been pointed out that multiple lesions in one or both breasts are likely to be benign solid tumors or a type of cystic

disease, and the rationale of simple excision in the former case and mastectomy in the latter has been explained. Personal experience in the immediate examination of breast tumors has demonstrated repeatedly the advantage of this form of coöperation between the surgeon and pathologist. An exploratory operation on the breast should not be undertaken unless one is fully prepared to perform a radical operation if malignant disease is found.

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THE CAUSE OF LOCAL REACTIONS FOLLOWING THE ADMINISTRATION OF STAPHYLOCOCCUS BACTERIOPHAGE*

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Some severe local reactions have been observed following the hypodermic injection of staphylococcus bacteriophage. Such reactions are controlled, to a certain degree, by size of dose and manner of injection, and according to past experience, do not contraindicate the use of staphylococcus bacteriophage. However, the usual appearance of definite local reactions affords one means of studying some of the constituents of staphylococcus bacteriophage. Therefore this study was undertaken to determine the nature of the reaction-producing substance in this preparation.

METHOD

The procedure involved the comparative measurement and evaluation of local reactions following the intradermal injection of 0.1 cc. or the various materials studied. The reactions were measured in millimeters and recorded as "circumscribed" or "diffuse," and also as "very red," "red," or "faint red" in intensity. Independent readings were made by two observers to exclude personal bias. While local reactions are commonly observed following injection of staphylococcus bacteriophage prepared by different laboratories, an effort was made in this study to avoid variation in the materials by using only the ingredients and products of one lot of staphylococcus bacteriophage. To avoid the possible sensitization of test subjects, a new group of individuals was used for each series of injections. When any comparison of reactions was attempted, all the substances under investigation were injected in the same subject at the same time to insure a comparative standard. In the beginning all reactions were read at 24, 48, and 72 hours, but it was observed that the

* This study was made possible through the kindness and cooperation of Dr. George F. Inch, Superintendent of the Ypsilanti State Hospital.

reaction reached the maximum at 24 hours and nothing could be learned from the fading residual. Therefore, in this investigation, the 24-hour reaction was the only one considered.

In order to conduct comparative studies of reactions under different conditions, it was necessary first to determine the reaction effect following the injection of staphylococcus bacteriophage. Fifty subjects were given 0.1 cc. of staphylococcus bacteriophage. Of these forty-seven showed a marked erythema which in some cases was almost inflammatory in character. To this group was also given simultaneously; 0.1 cc. of 1:10 dilution of the same bacteriophage, and forty-four positive results were secured, but the reaction was markedly diminished in size and intensity. Also simultaneous injection of 0.1 cc. of 1:20 dilution of staphylococcus

TABLE 1

	UNDILUTED BACTERIOPHAGE	1:10 DILUTION	1:20 DILUTION
	mm.	mm.	mm.
1	60 x 70 (very red)	16 x 40	10 x 14
2	45 x 60 (very red)	20 x 35	12 x 13

bacteriophage gave forty-four positive reactions but with a further diminution of the size and intensity of the reaction.

Table 1 illustrates two typical reactions from the series of fifty cases.

In normal individuals the size and intensity of reactions following the intradermal injection of staphylococcus bacteriophage is directly dependent on the concentration of the product.

The heat lability of the reaction-producing factor was then studied. Staphylococcus bacteriophage was boiled for half an hour with reflux condensation to avoid concentration, and the resulting product was given intradermally to fifty subjects in doses of 0.1 cc. Reactions resulted in forty-five cases but the size and intensity was much less than with the untreated phage. Bacteriophage was then subjected to autoclaving, the first portion being heated for 30 minutes at 15 pounds and the second portion, a total of 70 minutes, 30 minutes at 15 pounds, and 40 minutes

22 pounds. The first gave forty-four positive reactions which were much less marked than those of the boiled product. The second portion gave reactions in thirty-one cases, and the reaction in every case was much smaller and less intense than with the portions which had been subjected to less heat.

Table 2 shows three typical reactions from a series of fifty cases.

The reaction-producing substance in staphylococcus bacteriophage is sensitive to heat and the severity of local reaction is diminished in proportion to the degree of heat applied to the product.

TABLE 2

	UNTREATED PHAGE	BOILED PHAGE	AUTOCLAVED PHAGE	
			30 minutes, 15 pounds	30 minutes, 15 pounds 40 minutes, 22 pounds
	mm.	cm.	mm.	mm.
1	45 x 45 (red)	27 x 27 (red)	17 x 18 (red)	10 x 10 (red)
2	47 x 75 (red)	25 x 40 (red)	18 x 20 (red)	10 x 10 (faint)
3	25 x 45 (red)	20 x 30 (red)	12 x 15 (red)	10 x 11

TESTS WITH FRACTIONS OF BACTERIOPHAGE

For the purpose of this study, staphylococcus bacteriophage was considered to consist of (a) bouillon; (b) staphylococcus toxin; (c) disintegrated staphylococci (bacterial protein, endotoxin, and metabolic products); (d) bacteriophage (some substance or property that causes lysis).

Bouillon

The local reaction caused by Leibig's bouillon in 0.1 cc. doses was first investigated. The bouillon was given intradermally to a series of fifty individuals in 0.1 cc. doses of a 1:20 and 1:60 dilution, but no positive reactions were observed. The bouillon was then given intradermally in undiluted form to another series of fifty individuals with only one small, faint reaction. The bouillon was used as a control in one hundred and fifty other subjects and failed to cause any local reaction. Fifteen individuals were then selected and given 1 cc. of bouillon hypodermically with no

reaction resulting. Twenty-five new subjects were then given 5 cc. of bouillon hypodermically and all results were equally negative.

Bouillon is not a reaction-producing factor in staphylococcus bacteriophage.

Staphylococcus Toxin

In determining the role of staphylococcus toxin in the production of the local reaction, it was first necessary to separate this substance. This was done by growing *Staphylococcus aureus* in Leibig's bouillon for 48 hours under the same conditions as those followed in the preparation of bacteriophage and filtering the

TABLE 3
TOXIN

	0.1 cc.	1:10 DILUTION	1:20 DILUTION	BOILED ONE-HALF HOUR	AUTOCLAVED	
					30 minutes, 15 pounds	30 minutes, 15 pounds, 40 minutes, 22 pounds
	mm.	mm.	mm.	mm.	mm.	mm.
1	42 x 45	20 x 22	15 x 17	24 x 32	12 x 15	7 x 6
2	55 x 65	24 x 25	13 x 17	21 x 22	Negative	Negative
3	42 x 60	25 x 25	17 x 22	14 x 30	10 x 10	6 x 7

culture through a Pasteur filter. The resulting filtrate was a sterile mixture of bouillon and staphylococcus toxin. The absence of staphylococcus bacteriophage was proved by a series of lytic tests. Then a new series of fifty subjects was selected and the same procedure followed as with bacteriophage. The skin reaction from 0.1 cc. intradermal doses of this staphylococcus toxin approached in size and intensity the reaction caused by 0.1 cc. doses of staphylococcus bacteriophage. Dilution of the toxin caused an analogous diminution in the size of the reaction. The heated toxin solution followed the behavior of heated staphylococcus bacteriophage, that is, the more heat applied, the smaller and less intense the skin reaction.

Table 3 records three typical reactions from a series of fifty cases.

Staphylococcus toxin, in reaction-producing properties, behaves in practically the same manner as *staphylococcus bacteriophage* and is similarly influenced by dilution and by heat.

Disintegrated staphylococci

A 48 hour culture of *Staphylococcus aureus* was centrifuged and the bacteria separated from the broth and toxin. The bacteria were then suspended in a volume of physiological saline equal to

TABLE 4

MATERIAL	PERSONS TESTED	POSITIVE REACTIONS
Undiluted suspension.....	25	20
1:10 suspension.....	25	7
1:20 suspension.....	25	1
Boiled one-half hour.....	25	20
Autoclaved 30 minutes, 15 pounds.....	25	16
Autoclaved 30 minutes, 15 pounds; 40 minutes, 22 pounds.....	25	16

TABLE 5
BACTERIAL SUSPENSION

	SITE	1:10 DILUTION	1:20 DILUTION	BOILED ONE-HALF HOUR	AUTOCLAVED	
					30 minutes, 15 pounds	30 minutes, 15 pounds 40 minutes, 22 pounds
1	20 x 20	15 x 15	8 x 8	18 x 18	18 x 18	15 x 19
2	15 x 15	Very slight	Negative	11 x 11	10 x 11	11 x 11
3	Faint	Negative	Negative	Faint	Negative	Negative

the volume of the original culture. The growth was destroyed with 0.2 per cent tricresol. The resulting suspension was then allowed to stand for several days to allow disintegration of the bacteria and to insure sterility. This solution was tested and found to have no lytic principle. A series of twenty-five subjects were then selected and the diluted and heat-treated products of the suspension injected intradermally. The reaction from the suspension was usually a small area which was neither as inflam-

matory nor as definitely outlined as either the bacteriophage or toxin reactions. Dilutions of the suspension gave very few reactions which were usually of small size. Heat seemed to have very little effect on the bacterial protein and heat-treated solutions usually gave reactions on those individuals who reacted to the untreated protein.

The results are indicated in table 4.

Typical reactions from a series of twenty-five cases are indicated in table 5.

Bacterial protein from Staphylococcus aureus is a positive but minor factor in the production of local reactions.

Staphylococcus bacteriophage

It is impossible by the use of heat to destroy the lytic principle in staphylococcus bacteriophage without also affecting the toxin.

TABLE 6

	BACTERIOPHAGE			CONTROL		CONTROL BOUILLON
	0.2 per cent tricrosol	0.5 per cent phenol	Untreated	0.2 per cent tricrosol bouillon	0.5 per cent phenol bouillon	
	mm.	mm.	mm.			
1	40 x 50 (red)	40 x 50 (red)	30 x 55 (red)	Negative	Negative	Negative
2	35 x 47 (red)	35 x 48 (red)	30 x 50 (red)	Negative	Negative	Negative
3	47 x 60 (red)	40 x 65 (red)	45 x 65 (red)	Negative	Negative	Negative

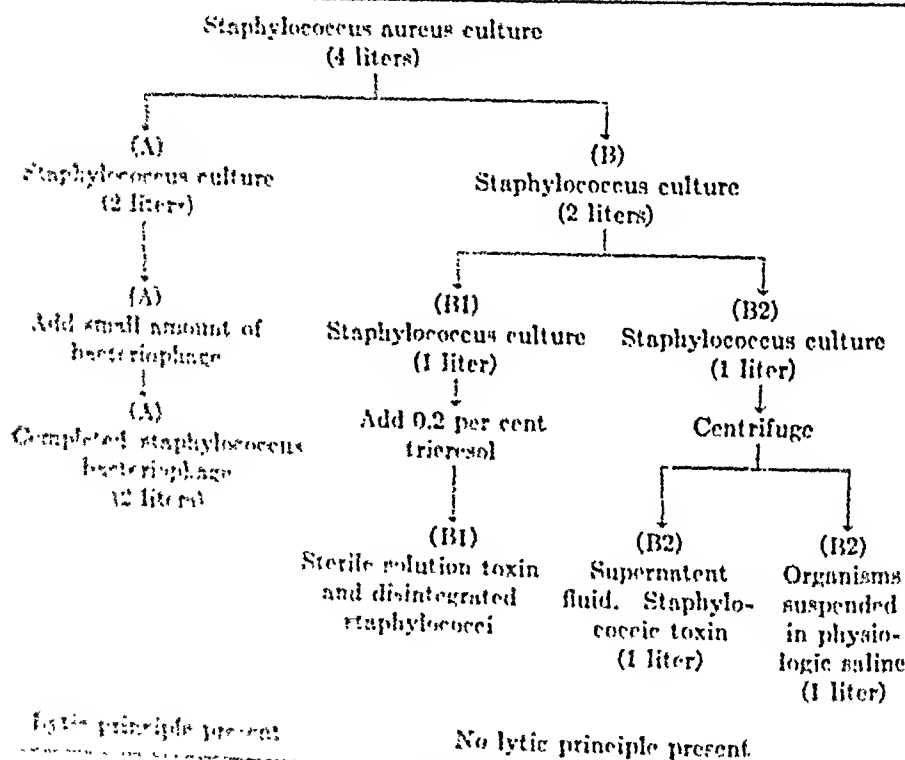
After a series of tests, it was determined that the lytic principle was destroyed by 0.2 per cent tricresol and also by 0.5 per cent phenol.

One lot of staphylococcus bacteriophage was divided into three parts. To the first portion was added tricresol (0.2 per cent); to the second portion was added phenol (0.5 per cent); the third portion was used as the control. The results of tests proved that lysis was absent in the first and second, but present and active in the third. Another series of twenty-five individuals was given 0.1 cc. doses of the three portions with control doses of 0.2 per cent tricresol bouillon, 0.5 per cent phenol bouillon, and unpreserved bouillon. The three phage solutions caused reactions

approximately equal in size and intensity. The controls were consistently negative.

Table 6 shows typical results from a series of twenty-five cases.

The chemical destruction of the lytic principle in staphylococcus bacteriophage does not diminish the size and intensity of the skin reaction.



flask "A" began to clear, culture "B" was divided into two equal parts (B1 and B2). To B1 was added 0.2 per cent tricresol to destroy growth, with the subsequent production of a bouillon solution of staphylococcus toxin and disintegrated staphylococci in a concentration equal to that in the completed staphylococcus bacteriophage. At the same time, culture B2 was centrifuged and the organisms thrown down. The supernatant fluid was removed and passed through a Pasteur filter to insure sterility. This fluid (1 liter) represented bouillon solution of staphylococcus toxin in a concentration equal to that in the completed staphylococcus bacteriophage. The organisms which had been thrown down were suspended in a liter of physiological saline, making a staphylococcus suspension of a concentration equal to that of

TABLE 7

	COMPLETED BACTERIOPHAGE (1)	STAPHYLOCOCCI TOXIN AND DISINTEGRATED STAPHYLOCOCCI (2)	STAPHYLOCOCCUS TOXIN (3)	DISINTEGRATED STAPHYLOCOCCI (4)	BOUILLON CONTROL
	mm.	mm.	mm.	mm.	
1	55 x 80 (red)	60 x 75 (red)	55 x 60 (red)	30 x 35 (red)	Negative
2	45 x 80 (red)	60 x 65 (red)	45 x 55 (red)	30 x 35 (faint red)	Negative
3	70 x 105 (red)	80 x 95 (red)	65 x 90 (red)	33 x 40 (red)	Negative
4	85 x 115 (red)	71 x 105 (red)	60 x 115 (red)	5 x 5 (faint red)	Negative
5	51 x 85 (red)	50 x 60 (red)	44 x 80 (red)	20 x 24 (red)	Negative

disintegrated staphylococci in the completed staphylococcus bacteriophage. Further growth was destroyed by 0.2 per cent tricresol.

Repeated tests for lysis showed that the lytic principle was absent in every fraction except in the staphylococcus bacteriophage. In the latter it was present and active. (See chart 1.)

This procedure gave four fractions: the concentration of each active principle was equivalent to its concentration in the completed staphylococcus bacteriophage. It was then possible to judge the reaction-producing properties of each component in comparison to each other and in comparison to the completed staphylococcus bacteriophage. Intradermal injections (0.1 cc. doses) were given to a new series of twenty-five individuals. Typical reactions from the series are shown in table 7.

From the above results it can be seen that the skin reaction of completed staphylococcus bacteriophage (1) is approximately equal to the reaction of combined staphylococcus toxin and disintegrated staphylococci; (2) the reaction to completed bacteriophage is approximately the sum of the reactions to staphylococcus toxin and disintegrated staphylococci; (3) the reaction of staphylococcus toxin approaches in magnitude and intensity the reaction of the completed bacteriophage; (4) the skin response to disintegrated staphylococci is small and slight in comparison to that in the case of staphylococcus toxin or completed bacteriophage.

The reaction caused by the intradermal injection of staphylococcus bacteriophage is practically equal to that produced by an equal concentration of staphylococcus toxin and disintegrated staphylococci from the same lot of product. The reaction is largely due to staphylococcus toxin but is in some degree dependent upon the presence of disintegrated staphylococci. The presence or absence of the lytic principle does not influence the frequency or degree of reaction.

SUMMARY

The present study has included the use of 425 individual test subjects who received 1625 intradermal injections, and forty individuals who were given hypodermic injections.

The results clearly indicate that the major reaction-producing factor in staphylococcus bacteriophage is the staphylococcus toxin present.

In consideration of these findings, the question arises as to whether the therapeutic value ascribed to staphylococcus bacteriophage when used hypodermically, is due in some measure to the presence of staphylococcus toxin.

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BLOOD IODINE STUDIES

IV. THE CLINICAL DETERMINATION OF IODINE IN BLOOD, URINE, AND FECES

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The interdependence of iodine and normal thyroid function is securely established. Extensive clinical studies are clarifying the significance of the blood iodine.^{6, 17, 22} Sturm's discovery,²² that the blood iodine is increased in hyperthyroidism, has been amply confirmed. There is an increased loss of urinary iodine in patients with hyperthyroidism.⁷ The relation of iodine metabolism to toxic goiter is receiving extensive attention. As a consequence the blood iodine, the urinary excretion of iodine, the iodine of the feces and the loss of iodine in the perspiration are assuming an increasing import in clinical investigation. As these investigations are becoming more widely known and appreciated, adequate clinical methods for the biochemical determination of iodine are being sought.

Quantitative methods for the determination of the minute amount of iodine normally present in living things have long been in use. These methods, however, have, in the past, proved to be successful only in the hands of a few experienced chemists. In fact, the early results of Chatin,^{3, 4} published between 1850 and 1876, were widely doubted and later forgotten. Contemporary chemists could not verify them. von Fellenberg's¹⁰ extensive and convincing studies have given them a belated confirmation.

Bourcet,² Blum and Grützner,¹ Hunter,¹¹ Kendall,¹⁴ von Fellenberg, Leitch and Henderson,¹⁶ Remington,²⁰ McClendon,¹⁸ Turner,²¹ Veil and Sturm,²² Lunde and Closs,¹⁷ Davis and Curtis,⁹ Karns^{12, 13} and von Kolnitz,¹⁵ have contributed to the development of methods for the determination of iodine in biological

substances. Recent progress is largely the result of von Fellenberg's contributions.

The primary purpose in making this particular investigation was to develop a method for use in the clinical laboratory. The method here presented is based upon the fundamental principles described by von Fellenberg. It makes possible the extensive clinical investigation of iodine metabolism. We have used it in investigating the blood iodine⁶ and in other studies.^{5, 7, 8} The volatility of iodine and the dissociability of its compounds make the analyses of such small amounts as are present in blood extremely difficult. Therefore, best results are obtained when the procedure is followed meticulously and is carried out in the minimum amount of time.

BLOOD

Blood is analyzed in 10 cc. duplicate samples. While carefully rotating a thoroughly cleaned 6 cm. nickel crucible containing an accurately measured portion of whole oxalated blood, 10 cc. of a saturated aqueous solution* of *iodine free* potassium hydroxide is added. The resulting dark mass of blood and KOH is boiled on a hot plate, or over a Bunsen flame, until the proteins are well hydrolyzed. When the mass has condensed to the point that it no longer foams vigorously, hydrolysis is usually complete. The crucible containing the homogenous mass is then placed in the muffle furnace set at 400°C. During this time and during the succeeding heating the crucible must be carefully watched in order to detect and control any excessive bubbling and frothing that occasionally may occur. An automatic temperature controller is necessary to maintain the muffle furnace constantly at the above mentioned temperature. After heating for one half hour the crucible is removed, cooled, and the mass thoroughly moistened with *iodine free* distilled water. The crusting on the walls of the crucible is well rinsed down. The water is best evaporated over a Bunsen burner. The crucible is then replaced in the muffle furnace. A slow stream of oxygen is admitted from a large cylinder to the combustion chamber of the furnace. The cooling and moistening process is repeated at twenty minute intervals. This procedure must be repeated until the charred mass is completely oxidized. When completely oxidized the original mass has a grayish appearance.

* We are indebted to Dr. D. Roy McCullagh of the Cleveland Clinic for his recent suggestion as to the use of large amounts of KOH in hydrolyzing the blood before it is ashed.

The water soluble salts are now extracted from this completely oxidized mass with three 15 cc. portions of iodine free distilled water. The solution is prevented from creeping over the edge of the crucible by greasing the lip with a thick vaseline. Each extract is filtered through the same Whatman No. 44 filter paper. This grade of filter paper, 9 cm. in diameter, has been found adequate. The filtrates are collected in another thoroughly cleaned nickel crucible. A nickel crucible is used in this stage because it will better withstand the later scraping and kneading of the dried salts. The filter paper is *always* allowed to drain between extractions. The transfer of the extract *must be done quantitatively*. After the last transfer has drained down, the inside of the funnel is sprayed with a few cubic centimeters of iodine free distilled water to insure complete solution and filtration of any remaining salts. The filter paper containing the insoluble oxides, and salts, is discarded. Repeated analyses of these filter papers, and of the contained oxides and salts, have shown that no appreciable amount of iodine remains in the filter paper after this thorough washing.

To make possible the alcoholic extraction of the iodine salts from the salt mixture, the combined distilled water extract from the filtrations is evaporated nearly to dryness, preferably on a steam bath, or in any way that does not cause spattering. The crucible is then removed and the remaining liquid carefully evaporated to a semi-viscid consistency over the low flame of a micro burner. The crucible should be constantly rotated during this process. This rotation minimizes spattering at the beginning of the evaporation process. At the end it facilitates the formation of smaller crystals. The presence of small crystals is necessary for the complete extraction of the iodine salts with alcohol. The optimum amount of evaporation is readily learned from experience. The point at which the solution begins to show crystallization, as evidenced by the formation of large bubbles, indicates an adequate amount of evaporation. The rotation must be kept up after the crucible has been removed from the flame and until the salts have completely crystallized into a firm grayish mass. This crystallization will occur at room temperature.

The iodine salts are now extracted from the mass of crystallized salts with alcohol. *Iodine free* ethyl alcohol is used. Ninety-five per cent concentration has been found to be optimum for the most complete extraction. Since the iodine salts are readily soluble in ethyl alcohol, they are quantitatively extracted with three 10 cc. portions. Only a minimum of other salts is brought over in the extract. It is important to minimize the amount of other salts in the extract because they may interfere with the titration. The original mass of salts, which should now be a thick homogenous white paste, is carefully kneaded in the alcohol with a chisel shaped metal rod. The entire inside of the crucible should be well scraped to insure complete extraction of any adhering particles. Each alcoholic extract is then quantitatively transferred to a 125 cc. Erlenmeyer flask.

This cumulative alcoholic extract is now evaporated to dryness on a steam bath. If the alcohol is not all boiled off the final titration color may be confusing. The crystallized salt mixture is then redissolved in *iodine free* distilled water. For the first addition 25 cc. are sufficient. Two drops of a 0.0007 molar methyl orange solution are now added. The pH is adjusted to about 4.0 by titrating the solution to a faint pink with 0.05 molar hydrochloric acid. Two cc. of a *freshly prepared iodine free* chlorine saturated water are now added. The pink color disappears on the addition of the chlorine water. The solution is now boiled gently over a Bunsen flame. The flask should be gently rotated to prevent undue bubbling and spattering. Boiling off about 45 cc. of water has been found adequate to insure complete removal of the excess chlorine gas. The original 25 cc. of water in the flask is boiled down to about 5 cc. Then 25 cc. more of iodine-free distilled water are added and boiled down to 5 cc. Before titration the concentrate must be cooled to about 25°C. The blue color of the starch iodine reaction does not appear unless the solution is well cooled. One small crystal of potassium iodide is now added. Too great an excess of potassium iodide causes a violet color to form. This color makes a recognition of the end point difficult. Six drops of a freshly prepared solution of soluble starch, (about 0.5 per cent), are now added. If iodine is present the characteristic blue color, commensurate in density to the amount of iodine that is free in the solution will appear within two minutes. The free iodine is then titrated with 0.001 normal sodium thiosulphate solution, using an especially devised micro-reservoir burette.¹⁹

URINE

Urine is analyzed in 25 cc. duplicate samples. While the crucible is being carefully rotated 1.5 cc. of saturated KOH are added. The KOH prevents loss of iodine when thoroughly mixed with the urine. The mixture is then carefully boiled to dryness. This requires about one-half hour. The resulting mass is heated in the muffle furnace for about twenty minutes at 400°C., without supplemental oxygen. The crucible is removed from the furnace, cooled, and the ash moistened with *iodine free* distilled water. It is then heated again in the furnace at 400°C., with supplemental oxygen, as in ashing blood. In one-half hour a nearly white mass of salts remains. The remainder of the process is identical with that used for blood.

FECES

The principles employed are identical with those as given for blood. Since there is a greater amount of organic matter present, it is necessary to add more of the saturated KOH solution. Therefore, 1.5 cc. per gram are used in order to obtain adequate hydrolysis. If the stool has a high fat content, alcoholic hydrolysis may be necessary. Before this is employed the mass should be well heated over a Bunsen flame until it is of even consistency. The crucible is

then placed on the steam bath and 25 cc. of 95 per cent ethyl alcohol are added. One addition is usually sufficient, although more may be necessary. The muffle furnace oxidation is carried out as with blood. This is not difficult if the material is well hydrolyzed and, consequently, homogenous. For extraction, the water and alcohol increments are increased proportionately to the amount of KOH employed. Titration is the same.

THYROID GLAND

Analyses may be accomplished by using the same procedure and the same proportion of reagents as for blood.

MILK

Analyses of the iodine content of milk are identical with those used for blood, except that alcoholic hydrolysis is employed as described under the method for feces.

WATER

Water is analyzed in the same manner as urine. Less time is necessary because of the minute amount of organic matter normally present.

REAGENTS

Potassium Hydroxide, saturated aqueous solution. This is made by saturating 1000 cc. of *iodine free* distilled water with "Kahlbaum" KOH.

Sodium Thiosulphate, 0.1 normal. Dissolve 24.832 gm. Merck's Blue Label, or P-W-R, crystalline salt in *iodine free* distilled water and make up to 1000 cc. This reagent should be kept in a dark bottle that is sterile⁹ as well as chemically clean. If the reagent is stored in a cool place the normality changes very slightly in months. The reagent is checked against the $\text{KIO}_3\text{-HIO}_3$ standard every two months. The 0.001 normal for the titration is made fresh each day by diluting 1 cc. of the 0.1 normal to 100 cc. in a volumetric flask.

Potassium Di-iodate, 0.1 normal. Dissolve 3.2496 gms. of $\text{KIO}_3\text{-HIO}_3$ in *iodine free* distilled water and dilute to 1000 cc. This reagent is used as the standard check for the sodium thiosulphate.

Ethyl Alcohol, 95 per cent. Made by diluting absolute alcohol with *iodine free* distilled water to 95 per cent.

Methyl Orange, 0.0007 molar. Dissolve 0.020 gm. of the sodium salt of dimethylamino-azo-benzene-sulphonic acid in 100 cc. of *iodine free* distilled water.

Chlorine Water. A saturated solution is made by bubbling chlorine gas from a cylinder into a convenient volume of *iodine free* distilled water until the solution is yellow. Chlorine gas manufactured by the Matheson Alkali Co., of East Rutherford, N. J., has been found to be iodine free.

Potassium Iodide. Merck's Blue Label, or P-W-R, is satisfactory.

Starch Solution, 0.5 per cent. One-half gram of soluble starch, prepared

according to Lintner, is made into a thin paste and added to 100 cc. of boiling iodine free distilled water. The boiling is continued for one minute after the addition of the starch paste or until the solution is clear. If the solution is not clear then the starch is not adequately dissolved, and therefore unsatisfactory.

Hydrochloric Acid, 0.05 normal. Merck's Blue Label is satisfactory. This reagent need be adjusted only approximately to the specified normality.

COMMENT

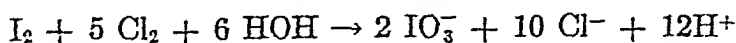
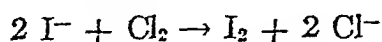
The chemical principles involved in the procedure are as follows: The oxidation of the blood in the presence of an excess of KOH

TABLE 1

DETERMINATIONS OF IODINE IN BEEF BLOOD MADE UPON THE SAME SAMPLE

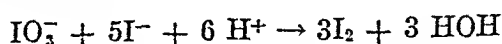
FIRST DAY	SECOND DAY	THIRD DAY
<i>gamma per 100 cc.</i>	<i>gamma per 100 cc.</i>	<i>gamma per 100 cc.</i>
14.4	12.3	16.1
14.8	14.6	15.2
14.4	14.6	16.3
14.6	15.7	16.5
14.4	14.4	16.7
15.8	13.0	12.5
12.5	15.3	16.3
13.1	13.4	16.9
		16.1
		15.6
Average... 14.3	14.2	15.8
Grand average for twenty-six samples.....14.8		

facilitates the formation of KI and possibly KIO₃ as rapidly as iodine is liberated from such other combinations as may exist. All the iodine present is eventually oxidized to iodate by means of chlorine gas. The oxidation occurs as follows:



When the iodate is reduced to iodine, by means of KI in an acid solution, the original value of the iodine is increased six-

fold.^{2, 23, 24} This reaction takes place rapidly and quantitatively at a pH below about 3.



Twenty-six analyses of the same sample of beef's blood from the slaughter-house, were made in three groups on consecutive days. The lowest value was 12.3, the highest 16.9, with an average of 14.8 gamma per cent for the entire series. The individual analyses are presented in table 1. When known additions of KIO_3 — HIO_3 were made to the blood samples the recovery varied from 80 to 94 per cent.

CALCULATION

$$1 \text{ cc. of } 0.001 \text{ N Na}_2\text{S}_2\text{O}_3 = \frac{126.993}{1,000 \times 1,000 \times 6} = 21.15 \text{ Gamma* of iodine}$$

$$(X) \text{ cc. of } 0.001 \text{ N Na}_2\text{S}_2\text{O}_3 \times 21.15 \times \frac{100}{n} \text{ Gamma of iodine per 100 cc. (or per 100 gm.)}$$

n = cc. of specimen, or gm. of specimen

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* Gamma = microgram.

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THE ACCURACY OF COMMON HEMOGLOBIN METHODS

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A survey of eighteen representative hospitals in this community was made recently to find out what methods of hemoglobin determination were in common use. Of the hospitals investigated, nine used the Tallquist method, five the Sahli, and four the Newcomer. In institutions where the Tallquist was used routinely, the Sahli or Dare method was performed in cases of anemia. Of all the instruments in use, only one had been calibrated against a more accurate method. The hemoglobin was reported in per cent, while in a few instances it was also recorded in grams per 100 cc. or in Sahli units. There was no uniform opinion among laboratory workers as to the relative accuracy of the instruments in use. A review of the literature did not reveal consistent and exact information concerning the comparative accuracy of the Tallquist, Dare, Sahli, and Newcomer instruments. The present study was undertaken to determine the average per cent error of these methods when performed under optimum conditions.

EXPERIMENTAL

In determining the accuracy of the common hemoglobin instruments, the calculation of the blood hemoglobin from the blood iron content (hemoglobin = 0.335 per cent iron) was used as a standard of comparison. This method^{4,7} has been shown to check closely with the oxygen capacity method, and has the advantage of being much simpler. Oxalated venous blood obtained from students, nurses and ambulatory patients was used for testing.

* With the technical assistance of Elizabeth Jane.

Duplicate determinations of the blood iron by the modified Wong¹² method and of the hemoglobin by the Newcomer, Sahli, and Dare methods were made on thirty-five samples of blood. An average of each two determinations was used for comparative purposes. The Tallquist method was included at first, but since blood hemoglobin values between 60 and 100 per cent could not be read with any degree of certainty, it was discarded as being inadequate for routine clinical work.

Iron content of blood

The blood iron was determined by the modified Wong method. It consists in the digestion of 0.5 cc. blood with concentrated sulphuric acid and potassium persulphate without heating, dilution,* precipitation of the proteins with tungstic acid, development of a color with potassium thiocyanate, and reading colorimetrically against a standard. All water was distilled in glass. Baker's C. P. potassium persulphate was used as this preparation gave no blank test for iron. Acid-washed filter paper was used throughout. This method was found to be simple, rapid and accurate, and is well adapted for use in the blood chemistry laboratory.

Newcomer

Determinations by the Newcomer method were made on a DuBoscq colorimeter fitted with a standard yellow disc and a blue glass filter (Bausch and Lomb). Artificial light was used for illumination. Two dilution pipettes were used and were found to check uniformly. Exactly thirty minutes were allowed between the dilution (1-500) and the colorimetric reading. No corrections were made for development of color.

Sahli

The Sahli determinations were performed with a Hellige type of instrument which contains two colored glass prisms in apposition to the diluting tube. Two pipettes and two cylindrical diluting tubes (used for all the tests) gave equivalent results. In the test, after adding blood to the small amount of $N/10$ HCl, exactly 10 minutes were allowed before diluting to the proper color. Since the color of the acid hematin continues to develop after this time, it is necessary to calibrate the instrument to a uniform length of time. In reading the end

* A precipitate that sometimes occurred at this point could be prevented if the blood and sulphuric acid were gently mixed and allowed to stand for two minutes instead of whirling as recommended by Wong. Determinations were more difficult to check when the precipitate occurred after water was added to the digestate.

TABLE 1
COMPARISON OF COMMON HEMOGLOBIN METHODS
 Per cent variation from the blood iron method

NUMBER	FLOOD IRON		NEWCOMER		SAHLI		DARE	
	Iron	Calculated hemo-globin	Factor: 1.047*		14.1 grams—100 per cent†		17.76 grams—100 per cent**	
			Hemo-globin	Variation	Hemo-globin	Variation	Hemo-globin	Variation
	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.	per cent	grams per 100 cc.	per cent	grams per 100 cc.	per cent
1	40.60	12.1	11.7	3.3	11.7	3.3	12.8	5.8
2	42.64	12.7	13.6	7.1	13.2	3.9	13.0	2.4
3	43.29	12.9	13.2	2.3	13.2	2.3	13.3	3.1
4	43.38	12.9	13.5	4.7	12.7	1.5	13.3	3.1
5	43.86	13.1	14.6	11.5	14.0	6.9	14.2	8.4
6	44.94	13.4	12.0	10.4	13.1	2.2	13.0	3.0
7	44.98	13.4	14.1	5.2	14.1	5.2	14.9	11.2
8	45.40	13.6	14.1	3.7	13.7	0.7	14.9	9.6
9	45.55	13.6	13.5	0.7	13.2	2.9	13.7	0.7
10	45.55	13.6	14.1	3.7	14.0	2.9	13.0	4.4
11	45.56	13.6	12.9	5.1	12.8	5.9	13.7	0.7
12	45.66	13.6	12.8	5.9	13.2	2.9	13.7	0.7
13	46.30	13.8	13.6	1.4	13.7	0.7	14.0	1.4
14	47.50	14.2	13.8	2.8	13.7	3.5	14.6	2.8
15	49.02	14.6	15.1	3.4	14.5	0.7	14.2	2.7
16	49.53	14.7	15.0	2.0	14.9	1.4	16.5	12.3
17	49.54	14.8	14.6	1.4	15.4	4.1	14.0	5.4
18	50.25	15.0	15.4	2.7	15.1	0.7	15.1	0.7
19	50.50	15.1	14.2	6.0	15.2	0.7	14.9	1.3
20	50.76	15.1	15.8	4.6	15.9	5.3	14.4	4.6
21	51.55	15.4	15.0	2.6	15.4	0.0	14.6	5.2
22	51.68	15.4	15.8	2.6	15.1	1.9	15.4	0.0
23	52.63	15.7	15.9	1.3	14.8	5.7	14.9	5.1
24	52.70	15.7	15.4	1.9	15.2	3.2	15.6	0.6
25	52.90	15.8	15.9	0.6	16.1	1.9	15.1	4.4
26	53.04	15.8	14.9	5.7	15.5	1.9	15.6	1.3
27	54.60	16.3	16.0	1.8	16.1	1.2	15.4	5.5
28	54.70	16.3	15.7	3.7	15.4	5.5	16.5	1.2
29	54.79	16.3	16.1	1.2	16.3	0.0	17.0	4.3
30	55.09	16.4	16.7	1.8	15.8	3.7	14.6	11.0
31	55.25	16.5	16.6	0.6	16.4	0.6	16.0	3.0
32	55.37	16.5	15.9	3.6	16.8	1.8	16.7	1.2
33	55.55	16.6	16.7	0.6	16.2	2.4	16.9	1.8
34	57.81	17.2	16.9	1.7	18.3	6.4	17.6	2.3
35	59.88	17.9	17.8	0.6	18.0	0.6	17.0	5.0
Average per cent variation.....				3.4		2.7		3.9

* Calibration by oxygen capacity method, factor : 1.039.

† Commercial calibration, 17.0 grams — 100 per cent.

** Commercial calibration, not known for this instrument.

point, the instrument was held at arm's length and rotated slightly until the color in the center of the diluting tube merged directly with the color of one of the yellow prisms. Daylight was used as a source of illumination. The intensity of the daylight seemed to make very little difference, although the readings were about 2 to 3 per cent higher by artificial light.

Dare

The Dare instrument used was fitted with a lamp and battery for illumination. Each determination consisted of an average of three readings on one pipette of blood. Attention has been called to the marked discrepancies in the width of the spaces between the glass plates of the pipettes,⁶ but the difference between the two pipettes used in these experiments was negligible.

The same set of hemoglobin values was used for calibration of each instrument and also for determining the average per cent

TABLE 2

VARIATION FROM BLOOD IRON IN THIRTY-FIVE HEMOGLOBIN DETERMINATIONS

METHOD	DETERMINATIONS LESS THAN 5 PER CENT	DETERMINATIONS LESS THAN 3 PER CENT
	<i>per cent</i>	<i>per cent</i>
Newcomer.....	89	54
Sahli.....	94	63
Dare.....	86	46

variation from the blood iron method. Determinations of the total blood iron and the hemoglobin by the Newcomer method were recorded in grams hemoglobin per 100 cc. while the Sahli and Dare readings were first recorded in per cent. The thirty-five average values were added for each of the four methods. Using the iron value as a standard, an average correction factor was calculated for the Newcomer method, and the grams hemoglobin equivalent to 100 per cent calculated for the Sahli and Dare methods. All figures were then converted to grams hemoglobin per 100 cc. according to these calibrations. These results are recorded in table 1 with their individual and average per cent variation from the blood iron method.

The average variation from the blood iron method was 3.4 per cent with the Newcomer, 2.7 per cent with the Sahli, and 3.9 per cent with the Dare method. Table 2 shows the propor-

tion of results with less than 6 per cent and less than 3 per cent variation. The Sahli instrument showed the greatest accuracy of the three when compared with the blood iron method.

In methods involving color matching, the personal equation may influence the accuracy of the results. In order to test this, each of two observers made a single determination on each of ten samples of blood, and the average difference of the two readings was calculated. One observer then made duplicate determinations on another group of ten blood specimens for comparison. Table 3 shows that two observers can check each other's determinations about as well as one observer can check his own. The larger error for the Dare method obtained by a

TABLE 3
AVERAGE DIFFERENCE BETWEEN TWO DETERMINATIONS EACH ON TEN BLOOD SPECIMENS

METHOD	TWO OBSERVERS	ONE OBSERVER
	per cent	per cent
Newcomer.....	0.8	0.6
Sahli.....	1.3	0.4
Dare.....	3.6	5.1

single observer may be related to eye fatigue in matching the colors. In general, it was possible to check results with greater accuracy with the Newcomer and Sahli than with the Dare instrument. Although the Newcomer instrument can usually be read within 1 to 2 per cent error, there were occasional blood specimens that had variations up to 10 per cent from the blood iron method, in spite of the fact that both blood iron and Newcomer determinations were rechecked several times. Wintrobe¹⁰ has also noted a similar discrepancy when comparing the Newcomer with the oxygen capacity method.

The results obtained represent the accuracy of common instruments when measuring hemoglobin values within the normal range.* It was our special purpose to find out the accuracy

* Normal range of blood hemoglobin;¹¹ Males—14 to 18 grams per 100 cc. Females—12-17 grams per 100 cc.

within this range, in order to know what significance to attach to variations in the normal and slightly subnormal levels. To check the accuracy of these methods for anemic blood specimens, series of determinations were made before and after diluting samples of blood with an equal amount of normal saline solution. The diluted blood gave the expected results by the Newcomer and Sahli methods, whereas by the Dare method the results were about 20 per cent too high. Consequently, if the Dare is calibrated for anemic blood specimens, the values obtained for normal blood will be too low. Such a relationship is shown in the figures given by Brown and Roth,¹ who also found the instrument to be quite accurate when measuring lower ranges of hemoglobin values.

DISCUSSION

In considering the accuracy of the hemoglobin determination in the average hospital laboratory, it seems that there is still need for improvement. The Tallquist instrument gives results that are too gross for careful clinical work. Other common methods that are not standardized are subject to considerable error. For example, different Newcomer instruments in the past have shown decided variations in calibration. The marked errors in the commercial calibration of Sahli instruments containing colored glass standards have been pointed out by Cullen.² Our results indicate that the Newcomer and Sahli instruments with glass standards are both satisfactory for clinical work when they are properly calibrated and the tests carefully performed. The Sahli method in our hands was found to be somewhat more accurate than the Newcomer method. The Dare method was less satisfactory because of (1) difficulty in checking determinations, (2) the degree of error when measuring values in the normal range, and (3) the variation in calibration at different hemoglobin levels.

Other simple methods for the determination of blood hemoglobin deserve greater trial. Recently, Karshan and Freeman³ have recommended the use of the acid hematin method of Cohen and Smith. It is shown that the simple iron method of Wong can be substituted for the oxygen capacity method in the prepa-

ration of the standard acid hematin. Osgood and Haskins^{5,6} have reported accurate results using a standard inorganic solution to be read colorimetrically or in the Sahli apparatus against an unknown acid hematin solution. It has the drawback of requiring a correction for the temperature of the standard. Sanford and Sheard⁷ have obtained good results with the use of a photoelometer. The chief objection to this method is its expense. Results reported with the Haden-Hausser⁸ method have been promising. It consists in the comparison of acid hematin with a colored glass standard. Of most importance in adopting any method for the determination of hemoglobin is knowledge as to the accuracy of the results obtained.

The desirability of expressing the hemoglobin content of the blood in grams per 100 cc. has been emphasized by many authors. It is evident that an expression in absolute figures becomes significant only when the method reaches a relative degree of accuracy. Such accuracy is obtainable with some of the common methods of hemoglobin determination.

SUMMARY

(1) The Newcomer, Sahli, and Dare methods of hemoglobin determination were calibrated by comparison with the blood iron method of Wong. Their relative accuracy was then calculated.

(2) The average variation of these methods from the blood iron method was 3.4 per cent with the Newcomer, 2.7 per cent with the Sahli, and 3.9 per cent with the Dare.

(3) It is concluded that the Newcomer and Sahli methods are sufficiently accurate for clinical work when they are calibrated and the tests carefully performed. The Dare method is less satisfactory because of the greater possibilities for error.

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THE PRESENCE OF ARSENIC IN THE BRAIN AND ITS RELATION TO PERICAPILLARY HEMORRHAGES OR SO-CALLED ACUTE HEMORRHAGIC ENCEPHALITIS*

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The presence of arsenic in any organ of the body in amounts greater than a mere trace can be considered abnormal. However, with increased usage, in the treatment of disease, of preparations containing arsenic, its presence in various tissues is to be expected in these cases. Arsenic in organic combination, and in various forms, exerts, in a few cases, a deleterious action on the hemopoietic system and, in still a smaller number of cases, a specific action on the capillaries of the white matter of the brain and of the spinal cord that leads to hemorrhage.

The condition in which miliary hemorrhages occur in the white matter of the brain and of the spinal cord following administration of some organic compounds containing arsenic frequently has been referred to as acute hemorrhagic encephalitis. The hemorrhages are limited almost exclusively to the white matter, the gray matter being practically unaffected. This finding is not usual in any recognized form of encephalitis. On histologic examination there is no evidence of inflammatory reaction. This was recognized by Globus and Ginsberg,¹ in 1933, when they suggested the term "pericapillary encephalorrhagica (due to arsphenamine)"; they thus excluded the inflammatory element and recognized the true cause of the lesion.

We are presenting four cases in which patients died following administration of organic compounds containing arsenic, as the

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result of pericapillary hemorrhage of the white matter of the brain (cases 1 to 4 inclusive, table 1) and a series of cases also in which organic compounds containing arsenic had been administered for the treatment of syphilis of the central nervous system but in which patients died from various other complications (cases 5 to 12, inclusive). These two groups of cases are compared with a group in which the subjects had ingested various inorganic arsenical compounds (cases 13 to 18, inclusive) and also with a group of five control cases in which there was no known history of administration of arsenic in any form (cases 19 to 23, inclusive).

The various types of complication resulting from administration of arsenical compounds may follow only two small doses, but more frequently they occur following several administrations of the drug. The complications listed in table 1 are seen following administration of all common types of organically bound arsenic used in the treatment of neurosyphilis. The time between onset of the complication, and death of the patient, varied from one to twenty-three days, and in cases of pericapillary hemorrhages of the white matter this time was decreased to from one to five days.

The age and sex of the patient was of no apparent significance. The determination of the arsenic content of the brain and liver was carried out by the electrolytic method of Gutzeit which was previously described by one of us (Osterberg²). Arsenic was more abundant in the white matter than in the gray matter of the brain in the four cases in which there were pericapillary hemorrhages (cases 1 to 4, inclusive). In these cases, the arsenic content of the liver varied greatly. In the group with other complications, the liver contained much larger amounts of arsenic than the brain. In cases 3 and 4, the patients suffered from and had been treated elsewhere for neurosyphilis, and the patient in case 2 had taken sulpharsphenamine of her own accord; consequently, it was impossible to determine the amount of arsenic these three patients had had.

At necropsy, the appearance of the brain in cases 1 to 4, inclusive, was most remarkable. The white matter of the entire cerebrum, midbrain, pons, cerebellum, and even of the pyramidal

TABLE I

CASE	AGE years	SEX	DIAGNOSIS	TYPE OF ARSENIC	CAUSE OF DEATH	ARSENIC, MCM. PER 100 GRAINS FIXED TISSUE	
						In liver	In brain
1	32	F.	Secondary syphilis	Neosarsphenamine 0.3 gm.; arsphenamine 0.3 gm.	Pericapillary hemorrhages of brain	0.125	0.120
2	30	F.	Syphilophobia	Sulpharsphenamine	Pericapillary hemorrhages of brain		0.200
3		M.	Intermittent hyper- tension	Unknown	Pericapillary hemorrhages of brain	Negative	0.180
4			Malignant hyperten- sion	Unknown	Pericapillary hemorrhages of brain	0.135	0.125
5	58	M.	Tabes dorsalis	Sulpharsphenamine; try- parsamide; neosarsphen- amine	Purpura hemorrhagica	0.53	0.300
6	48	M.	Neurosypilis	Unknown (eight treat- ments)	Thrombosis basilar artery and infarction of pons	0.360	0.094
7	49	M.	Neurosypilis	Arsenobenzol	Purpura hemorrhagica; aplas- tic anemia	0.375	0.080
8	53	F.	Latent syphilis	Sulpharsphenamine 2.3 gm. in 1923, 0.1 gm. in 1926	Acute hemorrhagic purpura	0.045	Negative
9	20	F.	Hereditary syphilis	Arsphenamine; neosarsphen- amine	Purpura hemorrhagica; aplas- tic anemia	0.110	Negative
10	45	M.	Neurosypilis, paresis	Tryparsamide	Hyperpyrexia	0.350	0.060
11	52	F.	Neurosypilis	Arsphenamine; tryparsa- mide	Subacute yellow atrophy of liver	Negative	0.060
12	38	M.	Neurosypilis, tabes	Arsenobenzol	Cirrhosis of liver	0.450	0.404
13	63	M.	None made	Inorganic (stomach wash- ings)	Chronic arsenical poisoning	0.061	0.350

14	50	F.	(Suicide)	Inorganic (found in stomach)	Acute gastritis (arsenic)	1.02	0.125
15	11*	F.		Inorganic	Acute arsenical poisoning and sepsis	1.7 0.250	Trace
16	4	M.	Acute poliomyelitis	Inorganic (accidental)	Acute gastritis (arsenic poisoning)	0.138	0.375
17	4	M.		Inorganic (accidental)	Arsenical poisoning	0.044	0.080
18			(Suicide)	Inorganic	Arsenic	1.00	0.25
19	11*	F.	Acute encephalomyelitis	Unknown	Acute encephalomyelitis (hemorrhagic)	0.740	Negative
20	76	F.	Traumatic contusion of brain	Unknown	Multiple hemorrhages of brain		Negative
21			Erysipelas	Unknown	Erysipelas and sepsis	Negative	Negative
22			Septic pharyngitis	Unknown	Septic pharyngitis	0.36	Negative
23			Bronchopneumonia	Unknown	Bronchopneumonia	0.45	Negative

* Months.

tracts of the medulla oblongata was infiltrated with innumerable small hemorrhages (fig. 1). Occasionally these small hemorrhages appeared to have fused and a large hemorrhage had occurred; microscopically, however, small hemorrhages were still recognizable. The gray matter did not reveal any hemorrhages, and those in the white matter were unusual. In the center of

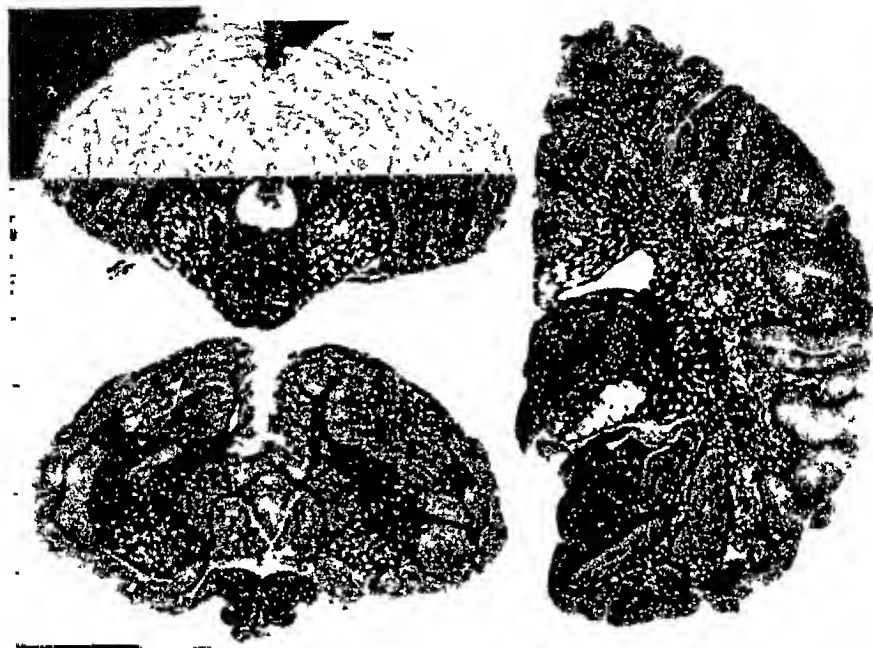


FIG. 1. PETECHIAL HEMORRHAGES IN THE WHITE MATTER OF THE CEREBRUM AND CEREBELLUM, EVEN IN THE PYRAMIDAL TRACTS OF THE MEDULLA

The gray matter of the cortex and basal nuclei have escaped the hemorrhages

each hemorrhage was a capillary filled with an eosin-staining, hyalin-like thrombus; the endothelial cells of the intima usually were prominent. Around this small blood vessel there was a zone of glial tissue free from erythrocytes, and around this zone of glial tissue was a wide ring of erythrocytes in an excellent state of preservation, without any sign of degeneration or of formation of blood pigment (fig. 2). In several regions in which

hemorrhages had fused, masses of polymorphonuclear leukocytes were present, but this seemed to us to be the result of destruction of brain tissue and not the cause of hemorrhage. In no other part of the white matter of the brain was there any evidence of acute or even of chronic inflammation.

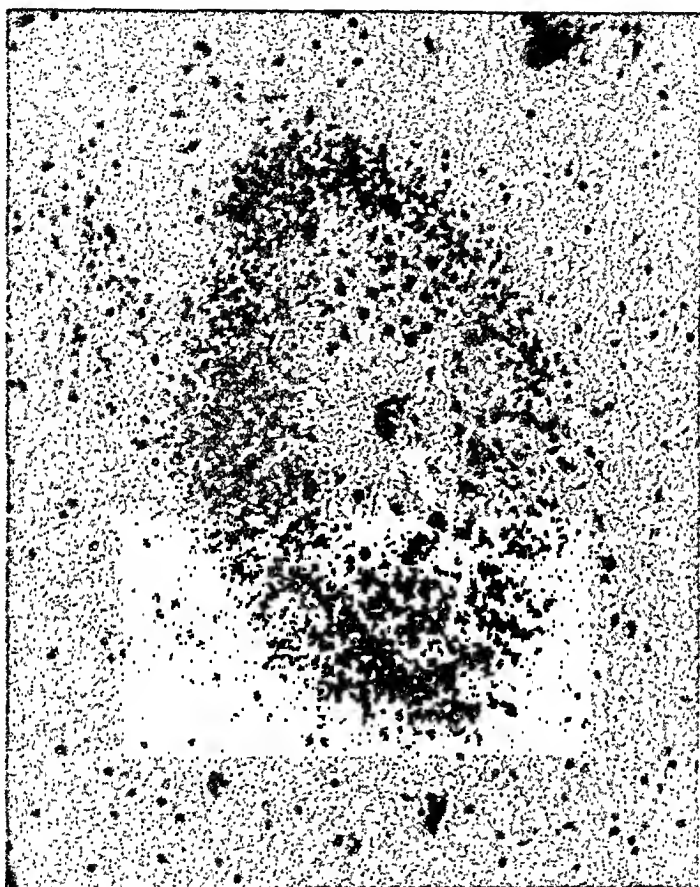


FIG. 2. TYPICAL PETECHIAL HEMORRHAGE

Thrombosed capillary in cortex, surrounded by a zone of glial tissue. The erythrocytes form a ring outside the glial zone (hematoxylin and eosin $\times 160$).

The arsenic content of the brain and of the liver in cases in the group in which the subjects had taken inorganic arsenic, either accidentally or with suicidal intent, were determined (cases 13 to 18, inclusive). In these cases hemorrhages in the central nervous system did not occur, yet the brain contained greater

amounts of arsenic than was found in the brain in cases in which organic arsenic had been administered or in which the patients had died from cerebral hemorrhage or from some other complication. The liver in these cases also contained varying, and usually large, amounts of arsenic.

The body of a child who had died of true hemorrhagic encephalitis was examined; the brain contained no arsenic, but the liver contained a large amount, the source of which could not be traced. Microscopic study of the brain in this case (case 19) revealed an acute inflammatory condition of the brain both in the gray and in the white matter. In the other four of these control cases (cases 20 to 23, inclusive) no arsenic was found in the central nervous system, but the amount in the liver varied considerably. The presence of arsenic in the liver cannot always be explained, but obviously there are numerous sources from which it might have been obtained.

SUMMARY

The condition known as acute hemorrhagic encephalitis frequently is not the result of an inflammatory condition, but may be due to the administration of organic compounds containing arsenic. The remarkable appearance of the brain and spinal cord is due to multiple capillary hemorrhages in the white matter of the central nervous system. Chemical determination gives evidence that arsenic is present in this tissue in relatively large amounts. When organically bound arsenic is administered in the treatment of neurosyphilis, it usually is demonstrable in the central nervous system in varying amounts. Inorganic arsenic, when ingested by accident or with suicidal intent, also accumulates in the central nervous system, but it seldom produces hemorrhages. We suggest that, in the presence of an unexplained gross hemorrhage or of multiple petechial hemorrhages in the white matter of the central nervous system, a chemical investigation for arsenic be carried out, since the presence of relatively large amounts of this element may explain the cause of the hemorrhages.

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BLOOD DYSCRASIAS

A SYMPTOM COMPLEX RATHER THAN A DISEASE ENTITY*

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Discussion has arisen within recent years with regard to the etiology and classification of the various so called blood dyscrasias. Ewing's¹ discussion indicated that infection and neoplasm seem to be intricately associated with the leukemias. Keim⁴ suggested that various forms of lymphadenosis listed under the diseases of lymphatic origin should be termed lymphoblastomas. In a former paper I⁵ expressed the opinion that leukemias and other blood dyscrasias showed evidence of being inflammatory rather than neoplastic in origin. An unusual disease of the bone marrow was recently reported² in which there were marked bone marrow changes, a symptomatic granulocytopenia, and a persistent increased temperature. Careful and continued observation, and a post mortem examination failed to reveal any evidence suggestive of any known clinical entity. Jaffe³ feels that each of these diseases is only a symptom complex produced by an outside stimulation or a faulty body reaction to such a stimulus.

Similar disease producing factors seem to elicit various responses. Blood pictures seemingly related to leukemia are often found in severe infections. The occasional blood findings of dye poisoning also simulate these leukemic phases of severe infection while the blood picture of granulocytopenia is frequently found following the use of any of the benzene ring derivatives. Both lymphatic leukemia and granulocytopenia not infrequently follow liver destruction caused by salvarsan. Granulocytopenia and aplastic anemia, too, seem to be definitely related and differ pathologically only in the extent of involvement found in the formative tissues.

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In the following case reports this intricate relationship of cellular pathology with the various apparent stimuli is illustrated.

CASE REPORTS

Case 1. Mrs. R., aged 43 years, with a history of phlebitis for two years, complained of pain in the upper left abdominal region. Examination revealed a huge spleen but blood findings did not show any evidence of leukemia. Because of the great discomfort, the spleen which weighed 1,475 grams (normal, 150 grams), was removed. Microscopic examination revealed definite evidence of leukemia. Two months later the leukocytes numbered 18,000 with 58 per cent lymphocytes and an occasional immature cell.

The case was diagnosed as aleukemic leukemia associated with an inflammatory process and an occasional immature cell found only after splenectomy.

Case 2. Mr. J. M., aged 70 years, complained of rheumatism beginning two years earlier. His leukocytes numbered 13,000 with no immature cells and his spleen was somewhat enlarged. He had been to the Mayo Clinic where his leukocytes ranged from 18,000 to 30,000 with no immature cells but a relative lymphocytosis of 45 to 63 per cent. At that time a possible lymphatic leukemia was considered. His condition became progressively worse, and just before death his leukocytes numbered 16,800 with 70 per cent lymphocytes and no immature cells. Post mortem examination revealed very marked leukemic infiltrations in all the organs.

A diagnosis of aleukemic leukemia associated with some inflammatory process showing no immature cells even up to time of death, was made.

Case 3. R. R. B., a boy aged 7 years, was suddenly taken ill, vomited, and was markedly cyanosed. His leukocytes numbered 14,000 with 68 per cent lymphocytes and an occasional immature cell. Because of the definite history a diagnosis of shoe dye poisoning was made; he recovered within a few days. His blood count gradually returned to normal.

This was a case of dye poisoning with a blood count simulating case 1 a definitely proved leukemia.

Case 4. Miss R., aged 23 years, became jaundiced after intravenous injection of neosalvarsan. The blood count revealed 32,000 leukocytes with 70 per cent lymphocytes, many of which were immature. Repeated leukocyte counts ranged between 30,000 and 50,000 with increasing numbers of immature lymphocytes. The patient failed to return but the blood findings justified the diagnosis of lymphatic leukemia following neosalvarsan injection.

A leukemic blood picture following administration of neosalvarsan, was diagnosed.

Case 5. E. M., a man aged 19 years, was given anti-luetic treatment, and after the second intravenous injection of salvarsan ran the usual course of a severe reaction. His leukocytes numbered 7,000 with 52 per cent neutrophils. Within a week he became jaundiced and his leukocytes kept dropping, ranging between 2,000 and 3,000 with granulocytes numbering 8 to 20 per cent. He was critically ill, but rallied and finally recovered completely. It may be questioned whether or not he had a true granulocytopenia but it is certain that there was a neutropenic phenomenon operating as part of the reaction to the neosalvarsan administration.

A neutropenic blood picture following administration of neosalvarsan was diagnosed.

Case 6. C. B., a man aged 27 years complained of a severe gangrenous sore throat. His leukocyte count numbered 3,000, only 20 per cent of which were leucocytes; a diagnosis of granulocytopenia was made. During the second week of illness the leukocytes rose to 7,000 with many immature leucocytes. The diagnosis was changed to leukemia. The day before death, which occurred at the end of the sixth week of illness, the total leukocytes were 22,000 with only 18 per cent mature and 50 per cent immature leukocytes. Post mortem examination of the spleen (weight, 2200 grams) and bone marrow revealed definite evidence of leukemia.

This proved to be a case of leukemia which began with typical symptoms of granulocytopenia.

CONCLUSION

The individual reactions in the form of various manifestations of so called blood dyscrasias which seem to be prompted by various stimuli in these cases indicate that each of these diseases is not in itself a disease entity but rather a symptom complex due to an outside stimulus or to some abnormal body constituent or a combination of both.

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EDITORIAL

ARTIFICIAL FEVER

It is apparently a human instinct to resist change, even though it is one of the certainties of life. Some misguided economists, whose theories were washed away by the economic tidal wave which inundated us some five years ago, have pleaded for a *moratorium on all scientific research for a decade*. Their plea is based upon the assumption that progress would cease during that time.

For centuries physicians regarded fever as an alarming symptom. Pathologists, following Virchow's teachings, ascribed various degenerative tissue changes to the effect of fever. Antipyretic drugs were almost universally employed to combat fever. From time to time, courageous observers ventured the unorthodox belief that fever was Nature's mechanism of defense against infection. In 1917 Wagner-Jauregg completely upset the teachings of centuries by demonstrating that artificially-induced fever was capable of overcoming the ordinarily disastrous effects of syphilis of the central nervous system. In the short period which has lapsed since this discovery, evidence has accumulated which makes it quite apparent that fever exerts an adverse influence upon the growth of bacteria, diminishes the potency of toxins, favors phagocytosis, and stimulates the development of immune bodies.

Wagner-Jauregg's success in malaria therapy of dementia paralytica was soon repeated by investigators in all parts of the world. The inherent dangers of engrafting one serious disease upon another as a therapeutic measure naturally led to a diligent search for a less hazardous method of producing the same effect. Comparable results were obtained by other workers following inoculations with the organisms of rat-bite fever and relapsing fever. Repeated injections of typhoid vaccine and other foreign

proteins appeared to be successful in those cases in which sustained high fever was induced. It became more and more apparent that simple fever production was the one factor common to all of these methods. These observations stimulated a demand for physical methods for artificial fever production.

The prolonged hot bath has been employed in the treatment of various infectious diseases since the time of the Greek priest-physicians. Laymen have clung tenaciously to their confidence in the curative merits of external heat. Schamberg and Tseng (1927), and Mehrtens and Pouppirt (1929) reintroduced the prolonged hot bath for thermotherapy. Rosanoff (1928) revived the outmoded hot air method. Neymann and Osborne, King and Cocke, and Whitney introduced the use of high frequency electrical currents (diathermy and radiotherapy). In the past four years many other types of apparatus have been devised.

While thus far most of the emphasis has been placed on the development of apparatus, those engaged in this field have become aware of great gaps in our knowledge of the indications for and the limitations of artificial fever therapy. History is repeating itself in the attempts of certain manufacturers to exploit the field by utilizing modern high-pressure sales methods. Some of the apparatus now available is inadequate and dangerous, and is sold to any physician without thought of adequate training of the supervising physician and his technical assistants. In the hands of physicians who have familiarized themselves with the medical and physical principles involved, with the assistance of intelligent nurse-technicians who have received special training, some of the machines now available are capable of producing sustained high fever (104° to 106.8°F. for five or more hours) with safety. The undertaking is in many respects comparable to a major surgical operation, particularly as regards the necessity for a careful diagnostic survey to determine eligibility and the constant attention to the patient during the long treatment. Neglect of contra-indications will lead to disaster. It is well to emphasize the obvious, but too often disregarded, fact that skill of personnel far transcends in value the perfection of matériel. Until further developed, artificial fever therapy should be re-

stricted to institutions. Otherwise an important adventure in therapeutics is almost certainly doomed to a period of discredit, similar to that which followed the introduction of roentgen-rays.

Fundamental research is urgently needed in this field. Little is known of the physiology of fever. Fever has never been adequately defined. The splendid preliminary studies which have been made by the small band of pioneers in this virgin field indicate that much is yet to be learned of the influence of fever on bacteria, tissues and body fluids. The thermal death-time studies made by Carpenter and his associates on *Neisseria gonorrhoeae* and *Treponema pallidum* indicate the great possibilities for the controlled application of fever therapy to the diseases caused by these organisms. Of equal importance, also, is the fact that these studies show that much of the available information regarding the thermal death-time of various bacterial species is erroneous. It is probable that many other pathogenic organisms will be destroyed or inhibited by sustained high temperatures.

In the fields of bacteriology, serology, immunology, hematology, biochemistry, and tissue pathology, as well as in the field of clinical medicine, the opportunities and the necessity for research are apparent. The clinical pathologist is a logical person to conduct such investigations. The present situation demands a shift of emphasis from machines to men.

—WALTER M. SIMPSON.

NEWS AND NOTICES

ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The Thirteenth Annual Convention of the American Society of Clinical Pathologists was a most successful occasion. It was held at Cleveland, Ohio, on June 8 to 11. A two-day program of scientific papers and exhibits contained a large number of excellent items. The round table discussion on Friday evening took the form of a Clinico-pathologic conference under the direction of Dr. P. F. Morse and was enthusiastically discussed. Dr. A. C. Christie read a paper on "Some Economic Problems Common to all Branches of the Medical Profession," a report of which will be published later.

The annual banquet was held on Saturday at which time the presidential address was read by Dr. A. G. Foord and Dr. M. T. MacEachern also spoke. The address of the evening was made by Dr. H. T. Karsner on "Medieval Guilds of Medical Interest." The address was followed by a program of entertainment.

Sunday and Monday were devoted to trips around the city and environs and included a visit to and luncheon at the Cleveland Clinic.

The business meeting will be reported in another issue of the Journal except for the following items:

The election of officers results in the following:

President-Elect: F. M. Johns.

Vice President: B. S. Kline.

Executive Committee (3 years): A. G. Foord, Kano Ideda.

Board of Censors: 3 years: Stanley Reimann, H. A. Heise.
1 year: A. H. Braden.

Board of Registry (3 years): W. E. King, Asher Yaguda.

A thorough revision of the constitution and by-laws was passed.

Dr. R. R. Kraeke received the Ward Burdick award.

Drs. Ludvig Hektoen and Otto Naegeli were elected honorary members and the following members were elected:

Horace Brockman Anderson, Johnstown, Pa.	Raymond Fridolph Peterson, Butte, Montana
W. V. Bergstrom, Binghamton, N. Y.	Clarence Carl Pflaum, Columbia, Missouri
John L. Beven, Baton Rouge, La.	Robert Burton Poling, Youngstown, Ohio
Paul Jean Breslich, Minot, N. D.	O. B. Pratt, Los Angeles, Calif.
Lewis Woodbridge Brown, Newark, N. J.	Ernest August Pribram, Chicago, Illinois
Frances Pullen Elliott, San Diego, Calif.	Otto Saphir, Chicago, Ill.
E. B. Erskine, Parris Island, S. C.	Edward Lowell Saylor, Akron, Ohio
Edward Fendrick, Irvington, N. J.	Joseph I. Schleifstein, Albany, New York
Roswell Schiedt Fidler, Columbus, Ohio	W. H. Seemann, New Orleans, La.
Wm. Freeman, Worcester, Mass.	Frederick Wm. Shaw, Richmond, Va.
Carl Goehring, Steubenville, Ohio	I. J. Silverman, New York, N. Y.
Samuel Alexander Goldberg, Newark, N. J.	Louis Alexander Soloff, Philadelphia, Pa.
H. Goldblatt, Cleveland, Ohio	Abraham Trumper, Montgomery, Ala.
Ernest Byron Hanan, Buffalo, N. Y.	Herman Henry Van Horn, Harrisburg, Pa.
Lewis Rowland Hill, LaGrange, Ill.	Stuart L. Vaughan, Buffalo, N. Y.
Robt. M. Holbach, Toms River, N. J.	Emmerich Von Haam, New Orleans, La.
Wilbur F. Keller, Oklahoma City, Okla.	T. T. Walker, Watertown, N. Y.
John L. Kestel, Waterloo, Iowa	Margaret Warwick, Buffalo, N. Y.
F. W. Light, Clarksburg, W. Va.	J. S. Weingart, Des Moines, Ia.
Dr. Wm. R. Mathews, Shreveport, La.	John Wenner, Allentown, Pa.
Perry J. Melnick, Chicago, Ill.	Corren Pinckney Youmans, St. Petersburg, Fla.
David Raymond Meranze, Philadelphia, Pa.	
John Davis Paul, Philadelphia, Pa.	

Attention of Clinical Pathologists is called to a recent decision by the supreme court re Granger v. Adson et al. (Minn. 250 N. W. 722).

Granger, a layman, conducted a so-called health audit service. For a fee, he undertook to examine urine and make blood pressure tests and to report the results. He employed a Dr. Grave, a licensed physician, to make the analyses and to report to him. Granger in turn passed on the report to the subscribers. The court held that Granger was as much practicing medicine in employing Dr. Grave to do the work for him as he would have

been if he himself had attempted to make the urine analyses and he did in making the blood pressure tests. "To pass on to the subscriber advice as to whether or not the tests indicated a normal or abnormal condition, and whether or not the subscriber should consult his physician or be content with the advice which Granger himself might give in regard to diet, exercise, and mode of living, was practicing medicine." "The law intends that the patient shall be the patient of a licensed physician, not the patient of a corporation or layman. The obligations and duties of a physician demand no less. There is no place for a middleman."

It is interesting to note that the court could not see any objection to the employment of technicians and other experts by physicians leaving the results of the work of the technician or expert to be interpreted by a physician as a help to diagnosis. The court held that Granger was practicing medicine in violation of the law.

This decision is of great importance to Clinical Pathologists for it should establish rather clearly the status of a technician, a bacteriologist, or chemist without a medical degree and not licensed to practice medicine. It clearly indicates that where such technicians or experts perform tests they must do so under the employment of physicians and that a physician must take the ultimate responsibility for the diagnosis, the report, and its interpretation.

BOOK REVIEWS

Urinary Analysis and Diagnosis by Microscopical and Chemical Examination. BY LOUIS HEITZMANN. 6th Ed. Pp. xxi + 366, 1934. Baltimore, William Wood & Co. \$5.00.

The text has been revised although the original general plan of the book remains the same. Tests are given which can be performed by physicians who do not have any but simple apparatus and modest laboratories. After introductory material, the chemical examination of urine is treated along rather orthodox lines. The exacting student will miss a critical evaluation of the many tests given under each heading and statement of the degree of sensitivity of these tests. The major stress in the book is on microscopical method, with which the second part deals. The author contends that with the use of higher magnification, one can tell the source of cellular materials and hence arrive at a diagnosis of the part infected and the nature of the lesion. The third part gives the application of the theory to clinical entities. The numerous illustrations are free hand pen and ink sketches, most of them very crude and not accurate as to size. Those illustrating parasitic forms are without value, and the discussion of *Trichomonas vaginalis* is inexcusable; that dealing with actinomyces is misleading and inaccurate. Dr. Dannreuther has contributed a chapter on functional kidney tests and the book closes with a chapter on hormone pregnancy tests.

The book may be of value to the clinician who lacks a well organized laboratory but the clinical pathologist will find it antiquated, limited, and below the standard of many modern texts.

Brucella Infections in Animals and Man. BY I. FOREST HUDDLESON. Pp. xvi + 108. New York, The Commonwealth Fund.

This is an important summary of methods of laboratory diagnosis in brucellosis. There is first an historical discussion of the genus *Brucella* which includes the general characteristics of the

three species. This is followed by a chapter dealing with methods of isolating the organism from cattle, from the milk of animals, from man, and from the tissues of animals, with details as to methods in which inoculation of guinea pigs is employed. The pathology of the infection in experimental guinea pigs and in natural infections of man and animals is discussed. The chapter following treats of the serological methods of determining infection with particular reference to agglutination and the shortcomings of these as diagnostic methods as applied to the disease in man. Details are given for preparation and interpretation of the nucleoprotein skin test and other such tests. The author then gives in detail his opsono-cytophagic test of the blood and its interpretation combined with the nucleoprotein skin test. The final chapter is concerned with methods of differentiating the species of the genus. A reference list of 188 items concludes the text. This is a useful and comprehensive manual for laboratory workers, and those interested in experimental phases of this subject.

THE ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE TEST*

WILLIAM G. EXTON AND ANTON R. ROSE

From the Laboratory and Longevity Service of The Prudential Insurance Company of America, Newark, New Jersey

Alimentary glucose tolerance tests are in general use because they tell more about the way an organism handles carbohydrates than other clinical methods. Like them, however, sugar tolerance tests also have limitations which are very real, and current clinical opinion concerning their diagnostic value may, perhaps, best be portrayed by a few quotations from contemporary authorities. Thus Joslin states: "Glucose and other food tolerance tests are often unreliable." Mosenthal says: "Increase of glycemia following the ingestion of sugar may be of value in diagnosing diabetes in some cases." Wilder writes: "Atypical responses which are difficult to evaluate are sometimes obtained with the dextrose tolerance test," and Rabinowitch points out: "With blood sugar time curves, as with other laboratory tests, a variety of conditions may be responsible for similar results."

Even more impressive than this unanimity of opinion is the prevailing diversity of practise in making sugar tolerance tests, and this involves not only laboratory technics. These are, in fact, unimportant in comparison with the existing differences in such practical details as preventing glucolysis, timing the tests, collecting the blood and urine samples, preparing and administering glucose and giving different amounts of glucose. Thus, the answers of one hundred clinical laboratories to a questionnaire about these details indicated in 1931 that it was very doubtful if any two of the laboratories were performing tests in exactly the same way.

The literature of sugar tolerance tests is extensive and dates

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

back many years, but there is nothing in it to suggest that differences of opinion concerning the physiology involved account for either the diagnostic limitations or variations in procedure. On the contrary, the principles are well understood and there is no disagreement about them. On the other hand, it is plain that the diagnostic limitations of sugar tolerance tests flow from their frequent failure to yield definite or dependable results. It is also clear that the diversity of practise is only evidential of individual efforts to secure results that are more specific in their meaning than atypical or doubtful blood sugar time curves which are at best difficult to interpret and often misleading.

FACTORS WHICH AFFECT THE SPECIFICITY OF SUGAR TOLERANCE TESTS

In a previous paper we³ have considered some of the reasons why the results of sugar tolerance tests so often lack specificity for the particular diminished carbohydrate tolerance of diabetes, and have also presented data of experiments intended to explore the possibilities of improving their specificity. In that paper we considered the test as a physiological experiment which at some definite time measured the difference between the rate at which ingested sugar enters the blood and the rate at which the same body either oxidizes, stores or excretes it. As such measurements are, of course, affected or vitiated by complicating conditions which are more or less uncontrollable, like diuresis, hydration, fatigue, emotion, digestion, et cetera, it was decided to confine attention to the chief factors in the practical application of sugar tolerance tests which might influence their specificity. From the work of previous investigators and our own experience it appeared that the chief factors are: (1) the time taken to make the test, (2) the antecedent diet or food habits of the subject and (3) the rate of absorption from the intestine of ingested glucose.

As usually performed, it takes two or more hours to make a sugar tolerance test, although it is clear that there are definite advantages in making the period of physiological experiments of this kind as brief as possible in order to exclude external and secondary influences on specificity such as emotional complica-

tions like anxiety and impatience, and physical discomforts like hunger, et cetera. For some years we have been giving a single 75 gram dose of glucose and limiting the test to two hours, counting a return of the blood sugar to within 20 per cent of the fasting value as the criterion for normals. In a very considerable amount of accumulated data no cases gave any more definite information at the end of three or four hours than at the end of two hours. In fact, the results of some cases seemed less obscure at the end of the shorter period. The patients preferred the shorter test in every instance, and the advantages of abbreviating the period have become still more apparent with the new procedure, which takes only an hour to complete.

Another and more important influence on the results of sugar tolerance tests is the antecedent diet or food habits of subjects. A number of authors have called attention to this factor, but none so vividly as Sweeney¹⁶ in this country, some of whose data are shown in figure 1. His curves show the average responses of groups of healthy young adults to glucose tolerance tests after living on exclusive antecedent diets for 48 hours. The curves are particularly suggestive when it is remembered that all of the subjects gave normal curves when tests were made after antecedent mixed diets and that reversals of the curves occurred with the same subjects on changed diets. It will also be observed that although all of the subjects were known normals, those who took nothing but water and those who ate exclusively fats or proteins gave misleading results, while only those normals who ate carbohydrates gave normal responses to the tests.

Sweeney postulated that these differences are due to variations in the stimulation of insulin and concluded:

The dextrose tolerance test as usually employed has been shown to be materially influenced by different antecedent foods or diets. . . . Some of the curves believed to be peculiar to different pathological conditions may result from the diets.

As opposed to Sweeney's results with the older test, the theoretical basis of the new test implies that antecedent diets or food habits do not influence the results. We therefore tested a number of subjects under the conditions of their accus-

tomed food habits in accordance with Nielsen's¹² conclusion that there should be no alteration of a subject's food habits prior to the test. It was also thought that information secured in this way would conform with the general run of actual conditions in clinical and insurance practise better than information obtained under the less usual conditions of depriving subjects of their accustomed food and restricting them to unaccustomed exclusive diets.

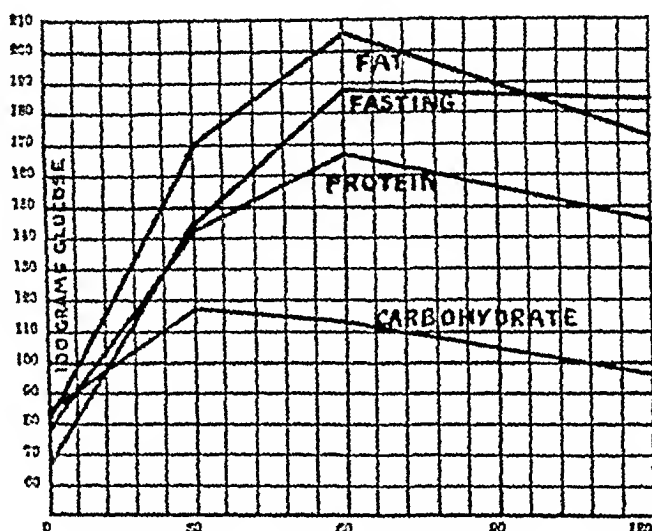


FIG. 1. Data of Sweeney showing effects of 48 hour exclusive antecedent diets on dextrose tolerance tests. Averages of four normal young adults on protein, five on fat, eight on carbohydrates and five on nothing but water. Only those on carbohydrates gave a normal response. In all of the figures, time in minutes is plotted on the abscissa and milligrams of sugar per 100 cc. of blood are plotted on the ordinate.

Two groups were made of sixty-five males and females, from eighteen to sixty-four years of age. Of these, some were known to be living on more or less exclusive diets. The first group consisted of six diabetics and forty-five non-diabetics. All of the members of this group were tested, not after the usual overnight fast, but at random times and regardless of what or when they ate. The second group consisted of fifteen diabetics, all of whom were tested, not at random times, but only after an overnight fast.

In both groups all tests gave perfectly typical results regardless of food conditions. Hence, antecedent food habits or diets do not affect the results of the new procedure.

Of all of the factors which influence the results of sugar tolerance tests the absorption of the ingested glucose from the intestine is by far the most troublesome. Woodyatt, Sansum and Wilder¹⁸ held that the irregularities inherent in absorption vitiated the results of alimentary sugar tolerance tests and proposed an intravenous procedure designed to avoid them.

Following their work, Beeler, Bryan, Cathcart, and Fitz,² after reviewing earlier experimental work, proposed "an improved alimentary glucose tolerance test" in which the usual procedure was modified by emptying the stomach with a tube an hour after the ingestion of glucose. By measuring the difference between the ingested glucose and the amount thus recovered from the stomach they aimed to ascertain the amount of glucose actually absorbed, and to interpret their results on that basis. They summarized their interesting experiments, which also cover some of the unavoidable complications of absorption, as follows:

Clinical observations emphasize certain obvious defects in the alimentary glucose tolerance test. . . . They have not received sufficient attention. The absorption of glucose from the intestinal tract is a complicated process. The rate of absorption varies with each individual within wide limits. . . . Glucose, in between 10 and 20 per cent solution, disappeared most rapidly from the stomach.

Their conclusions are of special interest when related to the concentrations of glucose employed in prevailing procedures which vary more on this than they do on any other point, although this is often obscured by insistence on other details, like the time allowed for drinking the glucose solution and its palatability.

It is typical of present practise to give 100 grams of glucose in 250 cc. of water. Some try to avoid the hypertonic effects of the 40 per cent solution by giving less glucose and others by giving more water. On this point a recent study of Magers¹⁰ confirms the findings of Fitz and his coworkers because no consistent differences appeared in the blood sugar curves of twelve subjects who were given 50 grams of glucose in 15 per cent solution, orally and

intraduodenally. In line with the experimental evidence that concentrations of glucose of about 15 per cent are least likely to impair results, the new test employs two 50 gram doses of glucose in 15 per cent solution.

THE PARADOXICAL LAW OF DEXTROSE

On the basis of animal experiments Allen¹ enunciated his paradoxical law as follows:

The paradoxical law of dextrose distinguishes sharply between diabetic and every type of non-diabetic animals. . . . Limits of tolerance in non-diabetic animals are all apparent, not real. The limits of tolerance in diabetic animals are real, not apparent. Just the opposite of the paradoxical law. . . . Whereas in normal individuals the more sugar given the more is utilized, the reverse is true in diabetes.

Allen was the first to suggest that his law might have diagnostic value, and Hamman and Hirschman,⁷ studied the effect on the blood sugar of the repeated ingestion of glucose. They included protocols which show the effects of the blood sugar of diabetics and non-diabetics when a similar dose of glucose is ingested three hours after a first dose. This effect has also been studied abroad under the name of the Staub¹⁶-Traugott¹⁷ phenomenon and other names, and the trend of these studies is illustrated in the recent text of Grafe,⁸ by blood sugar curves showing the effects of repeating a 20 gram dose of glucose one and a half hours after the original dose.

In an effort to differentiate between the glycosurias of diabetes and hyperthyroidism Rabinowitch¹² made a particularly interesting application of Allen's paradoxical law by giving diets of constant composition with reference to protein and fat, but increasing the amounts of glucose. According to his protocols 10 grams of glucose were given every hour for ten hours one day, 20 grams similarly the next day, and 30 grams on ten succeeding days.

It thus appears that under varied experimental and clinical conditions many observers have independently established the fact that normal human beings react to repeated doses of glucose with either hypoglycemia or little or no change of glycemia, while diabetics react with definite hyperglycemia. The underlying

physiology has been satisfactorily explained by MacLean and de Wesselow,³ and very well stated by Foster⁴ in summarizing his own experiments:

We interpret this fact as meaning that the first dose of glucose stimulates the insulin-glycogen mechanism to such activity that the normal organism is then able to deal with any amount of glucose without becoming hyperglycemic.

To this it may be added that diabetics react with distinct hyperglycemia because the insulin-glycogen mechanism fails.

We have, therefore, tried out the possibilities of the paradoxical law by varying the experimental conditions under which repeated tests were made on the same subjects, and have elsewhere presented a summary of these tests. They gave no evidence of being in any way affected by antecedent diets or irregularities of absorption, and very definitely indicated the practicability of adapting the paradoxical law to routine clinical use. They also enabled us to define optimum conditions, and formulate a simple and convenient procedure which has since been applied to more than four hundred individuals under usual clinical conditions.

THE ONE-HOUR TWO-DOSE TEST

Procedure

We perform the test by dissolving 100 grams of glucose in about 650 cc. of water. This solution is then flavored with lemon and divided into two equal doses containing 50 grams of glucose in about a 15 per cent solution, which are served cold and are easy to drink within a minute. We also keep at hand three containers with preservative against glucolysis^{14,8} for the blood specimens and three containers for the urine specimens, making it a point when collecting these to have subjects empty the bladder as completely as possible. The following steps are taken after a fast, preferably overnight:

- (1) Collect blood and urine (A) samples and give the first dose of glucose, allowing one to two minutes for its ingestion.

- (2) Thirty minutes after ingestion of the glucose, collect blood (B) sample and give the second dose of glucose, allowing one to two minutes for its ingestion.

- (3) Thirty minutes after the ingestion of the second dose of glucose, collect blood and urine (C) samples. The third urine container is given the subject for a sample of the urine next voided whenever a (post) sample is desired.

When the results of the foregoing procedure are plotted, the interpretation of the first part of the curve, that is, the part which

includes the original and the 30 minute samples, is exactly the same as the interpretation of the same part of the curve of the older procedure, and the same deductions are accordingly drawn from the blood and urine sugar values. The interpretation of the second part of the curve, the part which includes the 30 and 60 minute values, is not at all like that of the older test, and the following cases will serve to illustrate the criteria we use for interpreting the results of one-hour two-dose tests. Figure 2 shows the average responses of ten normal males and ten normal females,

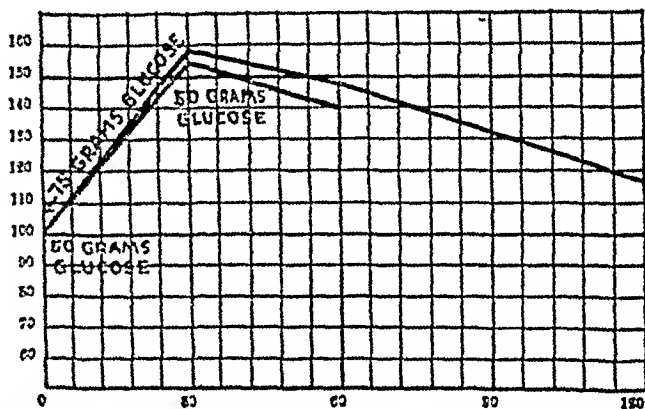


FIG. 2. Responses of ten normal males and ten normal females (ages 19-48) to one 75 gram and two 50 gram doses of glucose. Note the decline in blood sugar after the second dose of glucose; no sugar in urine.

19 to 48 years of age, to one 75 gram and two 50 gram doses of glucose.

Starting at 100 milligrams it will be seen that the first parts of both curves are practically the same. The rise in blood sugar following 75 grams is usually a little higher than that following the 50 gram dose of glucose. This tendency is, however, by no means invariable because many cases show the same or a higher rise in the blood sugar after 50 grams than they do after 75 or even 100 grams of glucose. Such instances are caused by more rapid and uniform absorption of the smaller amounts or concentrations of glucose, and an illustration of such a reversal of the expected results is seen in fig. 7.

The second part of the one-hour two-dose curve, that is, 30 and

60 minute values, indicates that normals respond to a second dose of glucose with a greater fall in blood sugar than that occurring during the same period after only a single dose of glucose. It is also characteristic of normals that there is no sugar in the urine. The typical criteria of normal responses to the one-hour two-dose test are, therefore: (1) a fasting blood sugar within the normal limits of the particular blood sugar method employed; (2) a rise in blood sugar which does not exceed 75 mgm. in the 30 minute sample; (3) the blood sugar in the 60 minute sample is less, the same, or does not exceed the 30 minute sample by more than 5 mgm., and (4) all urine samples are negative to Benedict's test.

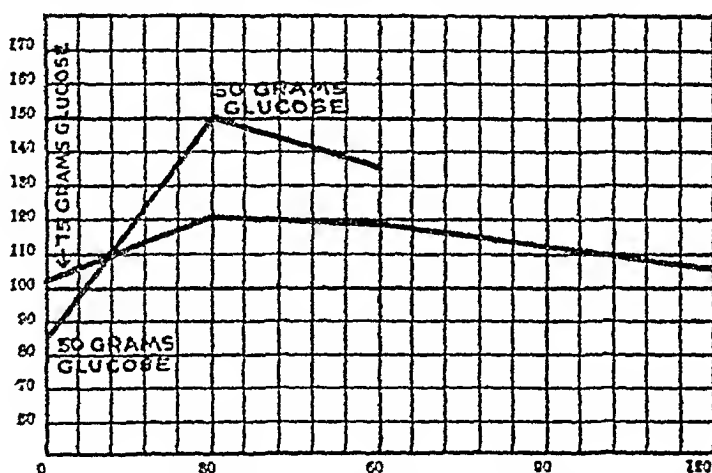


FIG. 3. CASE 1. Illustrates a typical flat curve of older test and typical normal result of new test.

The extent to which certain results detract from the clinical value of sugar tolerance tests was indicated by the quotations from authorities in the introductory part of this paper. Some of these questionable results occur in the form of curves having delayed peaks or curves with plateaux or curves which tend to flatness. We have had a number of these cases. Although the responses to the older test were doubtful or misleading, all of them gave perfectly typical responses to the one-hour two-dose test. An obvious explanation of these discrepancies is offered by the differences observed after introducing glucose into the duodenum at various rates which Hale-White and Payne⁶ have interpreted

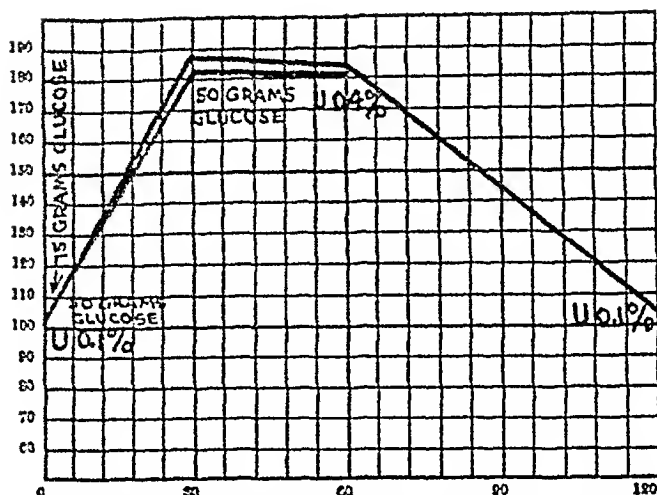


FIG. 4. CASE 2. Has sugar in urine occasionally. Illustrates so-called plateau curve of old test. By the new test, the typical normal shape of the curve, the higher than normal level of the blood sugar, and the concentration of sugar in the final urine indicate a mild alimentary glycosuria. (Case of Dr. Wm. Tilton.)

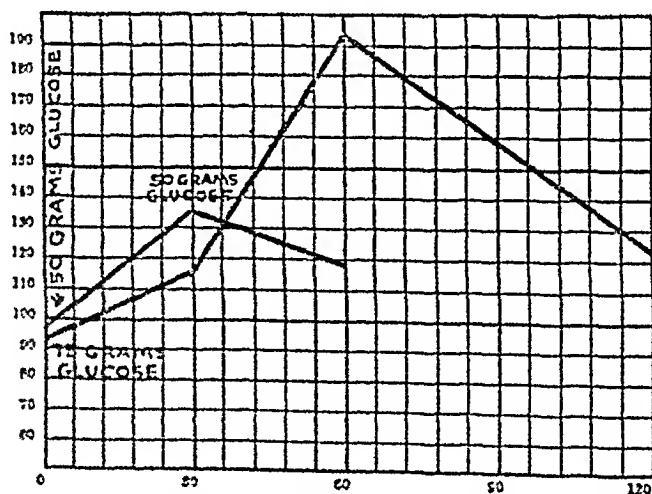


FIG. 5. CASE 3. Illustrates delayed peak curve of older test in a normal subject, probably due to delayed absorption of glucose. No sugar in any of the urine samples but blood sugar values of older test make the diagnosis doubtful. The result of the new test is typically normal.

as meaning that the emptying rate of the stomach influences the glycemic period. Cases illustrating flatness, a plateau and a delayed peak, in the curves given by the older test, but perfectly typical responses to the new test are seen in figs. 3, 4 and 5.

Unlike the preceding curves, those in fig. 6 represent the characteristic responses of a patient with mild diabetes. The responses of another mild diabetic in fig. 7 show a perfectly typical result of the new but an atypical result of the older test. In com-

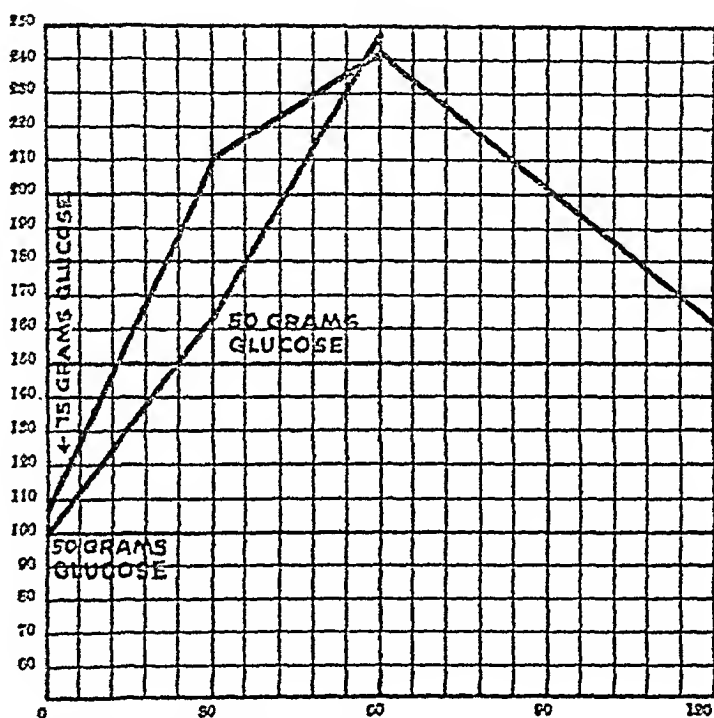


FIG. 6. CASE 4. The steep climb of the blood sugar curve after the second dose of glucose is typical of diabetes. (Case of Dr. W. R. Tilton.)

paring the responses of these diabetics to the older tests at the end of the two hour periods it is evident that the hyperglycemia in case 5 (fig. 7) is increasing when case 4 (fig. 6) is decreasing. In case 4 it is also evident that the rise in blood sugar after 75 grams of glucose is greater than that after 50 grams, as it logically should be, while in case 5 the blood sugar rises higher after the lesser amount of glucose, contrary to theoretical expectations. This case also illustrates how some diabetics, as well as normal

persons, respond to the older test with atypical results due to irregular absorption while the results of the new test are perfectly typical.

In the one-hour two-dose tests of both mild diabetics it is apparent that the first parts of the curves are similar in trend to those of the older tests but that the latter parts which show the effects of the second doses of glucose are distinctly different. Here the steep climb of the blood sugar characterizes diabetes, and sharply distinguishes it from all non-diabetic conditions in accordance

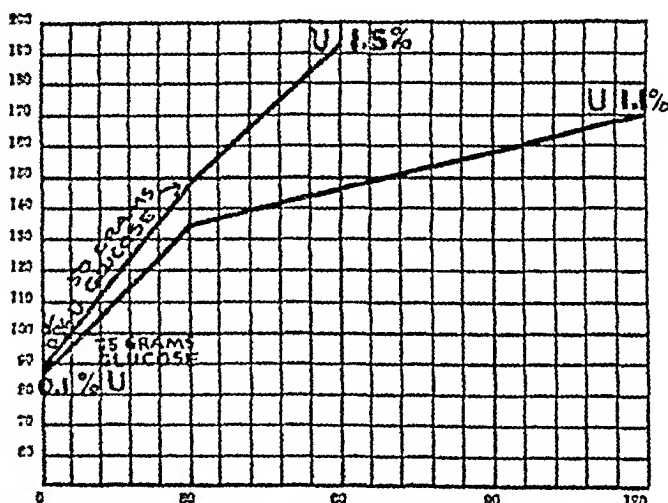


FIG. 7. CASE 5. Mild diabetes showing atypical result of older test due to irregular absorption and typical result of the new test. (Case of Dr. E. S. Dillon.)

with the paradoxical law. In both cases it will also be noted that there is no sugar in the urines from the fasting patients, but sizeable concentrations in the samples voided after ingestion of glucose.

In more severe or advanced diabetes the responses to the one-hour two-dose test are in a general way the same as the responses of mild diabetes, only the sugar values run higher in all the blood and urine samples of the severer cases. The tendency for the difference between milder and more severe diabetes to be quantitative is apparent in the curves of fig. 8 which shows the results

of old and new tests in a case of more severe diabetes. From these illustrations it is evident that *the criteria for determining diabetes in the one-hour two-dose test are a more or less steep rise of not less than 10 mgm. of blood sugar in the 60 minute sample following the*

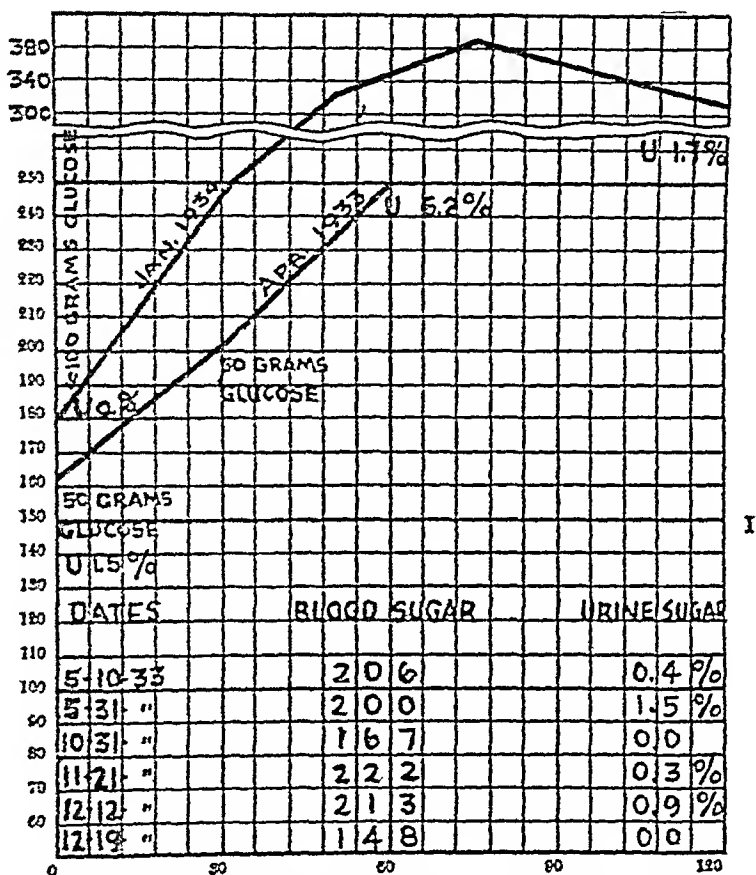


FIG. 8. CASE 6. Illustrates typical results of both tests in more severe diabetes, i.e., same criteria as mild diabetes but higher sugar values related to the severity of the disease. Case also illustrates tendency to high and changing renal threshold for glucose. (Case of Dr. H. O. Mosenthal.)

second dose of glucose and the relation of blood and urine sugar values to the severity of the disease.

In some cases of diabetes the renal threshold for glucose changes from time to time as it does in non-diabetic derangements of carbohydrate metabolism. Case 6 shown in fig. 8 also illustrates such an instance. In some of the cases the blood sugar values

may run very high when the urine contains little or no sugar. These tendencies are not characteristic of any particular condition but more likely associated with complications or with derangements of carbohydrate metabolism which may be secondary to some clinical condition other than diabetes.

A wrong diagnosis of diabetes is made more often in cases of renal glycosuria and pentosuria, especially the latter, than in other persistent glycosurias. Furthermore, it is not at all exceptional to find cases of pentosuria also incorrectly diagnosed as renal glycosuria. As both of these conditions give similar responses to sugar tolerance tests, this mistake can hardly be avoided unless the precaution is taken to identify the sugar found in the urine. In order to avoid unnecessary alarm and treatment it is particularly desirable to do this when the concentrations of sugar are small.

The diagnosis of renal glycosuria rests upon criteria which are still subjects of considerable difference of opinion and practise. Perhaps the strictest criteria are those of Joslin and his associates, and fig. 9 shows the results of old and new tests on one of their cases of renal glycosuria, (No. 2279 of Marble's¹¹ report). It should be noted that different blood sugar techniques were employed in making these curves and that the new test was made several years after the older one. In the one hour curve it is of interest to find the blood sugar after the second dose of glucose in the borderland between normal and diabetic values. While other cases of renal glycosuria have shown the same tendency, more cases show blood sugars like normals. The cases of pentosuria behave in the same way, and it is, of course, characteristic for all urine samples to show sugar in both renal glycosuria and pentosuria. *The criteria of the new test for renal glycosuria are, therefore: blood sugars which follow the normal course, or in any event never reach the diabetic level, and sugar in both urine specimens.*

In addition to the persistent glycosurias, there are intermittent glycosurias which may or may not be of diabetic origin. In these cases the sugar tolerance test is the only means at our disposal for distinguishing between diabetes and alimentary glycosuria. In the cases of alimentary glycosuria the fasting blood sugar is within

normal limits like some of the diabetic cases. Following the first dose of glucose the 30 minute blood sugar in some of these cases may remain within normal limits, but like the diabetics it more usually exceeds these, often very considerably. After the second dose of glucose, however, a very distinct difference appears between the alimentary glycosuria and cases of diabetes. The blood sugar distinguishes sharply between them because it rises characteristically in diabetes and falls in alimentary glycosuria as in normals. In both conditions the final urine samples show more

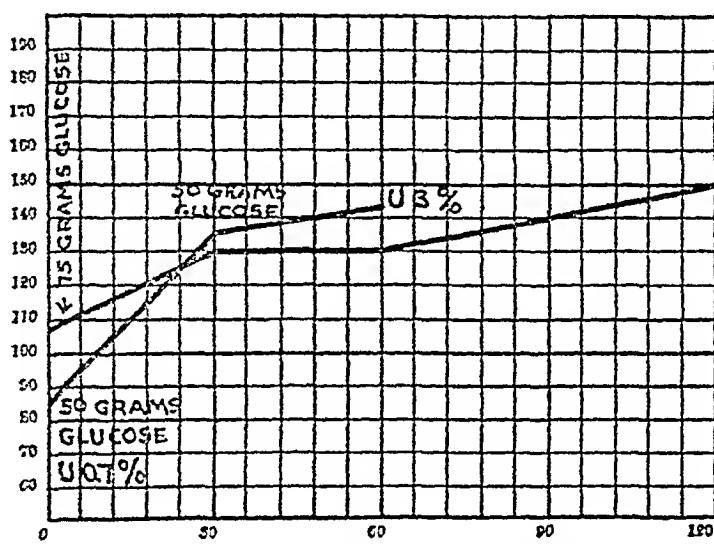


FIG. 9. CASE 7. Renal glycosuria. The blood sugar does not reach the diabetic level after a second dose of glucose. (Case of Drs. Joslin and Marble.)

or less sugar, depending upon the threshold of the individual. Figure 10, when compared with the diabetic curves in figures 6, 7 and 8, shows the characteristic distinction between alimentary glycosuria and diabetes. It should also be compared with the milder case of alimentary glycosuria with plateau curve of the older test seen in fig. 4. *The criteria of alimentary glycosuria are, therefore: a sugar-free urine after fasting with sugar in the final urine and blood sugars that follow the normal curve even when the level is higher than normal.*

While the foregoing conditions represent types of glycosuria in

which the carbohydrate metabolism is of special clinical interest, there is a variety of other conditions which secondarily or accidentally affect carbohydrate metabolism. Some of them are associated with glycosuria, generally intermittent in character; others are not associated with glycosuria. In some of the cases the disturbance of carbohydrate metabolism is temporary or trivial, as in colds and other infections or toxemias, menstruation, et cetera, and it is, of course, advisable not to make sugar tolerance tests at

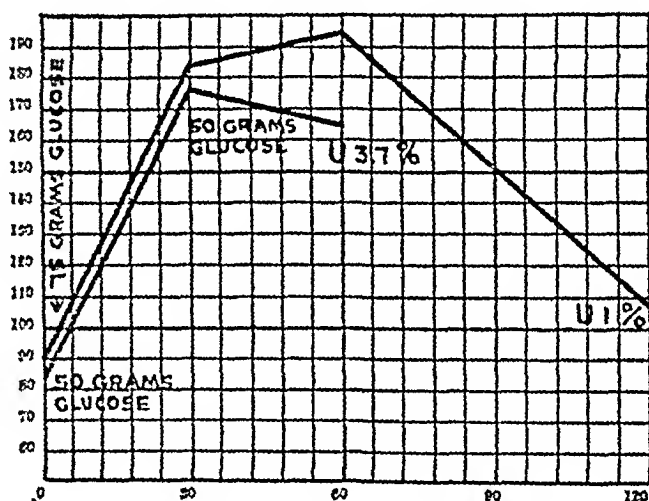


FIG. 10. CASE 8. In alimentary glycosuria there is no sugar in the urine when patient is fasting but definite amounts after ingestion of glucose. The new test sharply distinguishes between this condition and diabetes by the fall in blood sugar after the second dose of glucose instead of the rise characteristic of diabetes. Compare with fig. 4 showing a similar but milder case. (Case of Dr. P. V. Reinartz.)

such times. In other cases the glycosuria depends on more fundamental disturbances of carbohydrate metabolism which must be differentiated from diabetes, such as those associated with endocrine and psychic conditions. Cases illustrating equivocal results of the older test in thyroid and emotional conditions but unequivocal results of the new test are shown in figures 11 and 12.

Up to the present time not a single instance has been encountered in which the new test disagreed with the older when the

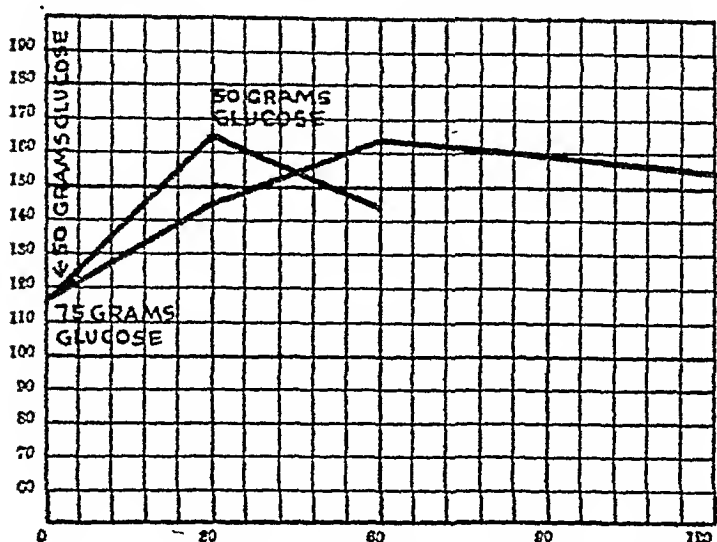


FIG. 11. CASE 9. Nervous and irregular menses. Under treatment for thyroid trouble. Sugar in urine occasionally, the new test definitely excludes a diabetic tendency but the older test does not. (Case of Dr. M. K. Smith.)

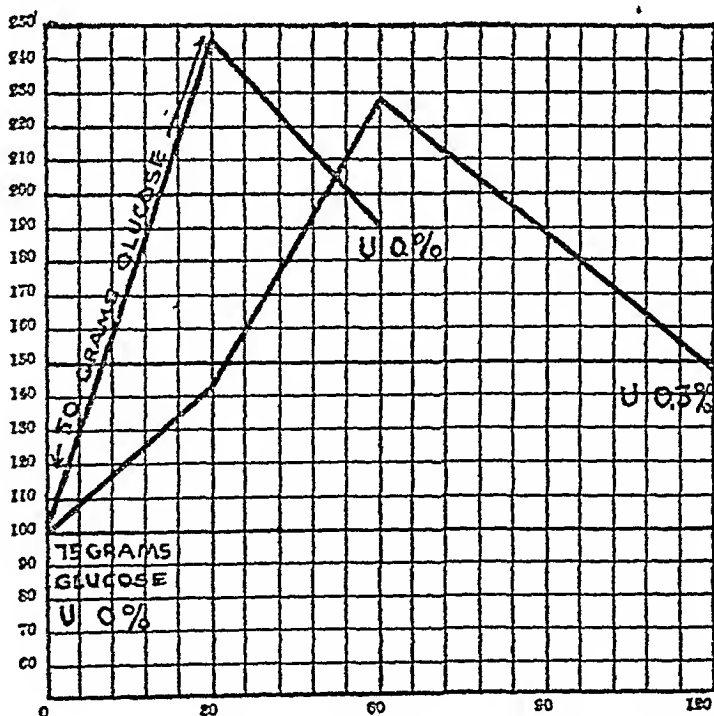


FIG. 12. CASE 10. Illustrates psychic effects on sugar tolerance tests. In this case, emotional disturbance related to secret marriage and early pregnancy. The older test indicates diabetes but the new test does not. (Case of Dr. W. R. Tilton.)

results of the older test were satisfactory. On the other hand, many cases gave doubtful or misleading responses to the older test when results of the new test were consistent and specific.

SUMMARY

Some of the factors which influence the results of alimentary glucose tolerance tests have been discussed in connection with the development of a new procedure designed to avoid them.

A one-hour two-dose test based on Allen's paradoxical law of dextrose has been described and its diagnostic criteria illustrated by clinical applications of older and new tests to more usual types of glycosuria.

Besides its greater convenience, all of the available evidence indicates that results of the new test are more specific and reliable.

We express our appreciation of the careful technical work of Miss Cecile Blacker in executing these studies.

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SOME MEDICOLEGAL ASPECTS OF ISO-AGGLUTININS*

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Although the discovery of blood groups, by Landsteiner in 1900, has proved its value in medicolegal cases abroad, the legal profession of this country has failed properly to recognize its possibilities. In Europe the reliability of blood grouping has been established by more than 10,000 paternity cases alone, yet the literature of this country fails to record a single case in which evidence obtained by blood grouping was admitted as competent testimony in an American court, and in which a judge handed down an opinion concerning the value of such evidence.

The importance of the universal recognition of the laws governing the heredity of the blood groups can be realized when one considers the results of more than 5000 cases of disputed paternity, reported by Schiff⁷ of Berlin in 1929. Had all of the accused men been innocent, only 16 per cent could possibly have proved non-paternity by the methods in use at that time. Actually, the innocence of half that number, or 8 per cent, was definitely established, from which it follows that one-half the total accused group in reality was not guilty. Therefore, assuming the same distribution of guilt and innocence in European and American groups, we must conclude that one-half the defendants in fornication and bastardy cases are falsely accused of at least half of the charge. Also since accusations of bastardy are usually impossible to disprove by the methods now in use in this country, convictions are almost certain to follow, or if cases are settled out of court in order to avoid scandal, a legalized form of blackmail is possible. Furthermore, the knowledge of blood groups has solved the problem of mixed children, and also furnished valuable clues in criminology.

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

THEORY

In 1910 von Dungern and Hirschfeld² concluded that the blood groups were inherited as two independent pairs of Mendelian factors. They believed that the iso-agglutinogens A and B were dominant to the corresponding iso-agglutinins a and b. On the basis of this theory they correctly assumed that the factors A and B could not appear in a child unless they were present in at least one of the parents. This theory of independent pairs of factors is untenable because it does not explain the percentages of the blood groups of children born of parents of known blood groups, and, furthermore, erroneously admits the

TABLE 1

GROUPS OF PARENTS	GROUPS OF CHILDREN POSSIBLE	GROUPS OF CHILDREN NOT POSSIBLE
O × O	O	A, B, AB
O × A	O, A	B, AB
O × B	O, B	A, AB
A × A	O, A	B, AB
A × B	O, A, B, AB	
B × B	O, B	A, AB
AB × O	A, B	O, AB
AB × A	A, B, AB	O
AB × B	A, B, AB	O
AB × AB	A, B, AB	O

possibility of group O offspring from an AB parent. Bernstein¹ proposed a theory which agrees with the actual facts of heredity, namely that there are three multiple allelomorphs A, B, and O. Since A and B act as dominants, and O as recessive, table 1 illustrates the possibilities of inheritance of blood groups.

Although some investigators have occasionally found exceptions to the above, these can be attributed to technical errors or to illegitimacy. It seems that the advice of Ottenberg⁶ has not always been heeded, namely, to acquaint the mother with the purpose of the test so that she can object if she has reason to be in doubt as to the actual fathers of her children. By following Ottenberg's plan I have been able to determine the blood groups

of 246 "certified" parents and their 259 children, and it will be seen that no exceptions to the Bernstein theory are found (table 2).

If, in addition to the above hereditary factors, A and B, the recently discovered agglutinogens M and N described by Landsteiner and Levine⁴ are also considered, the medicolegal value of blood grouping is about doubled.

TABLE 2

MATING	NUMBER OF FAMILIES	CHILDREN IN GROUP			
		O	A	B	AB
O × O	18	33			
O × A	46	36	42		
O × B	9	10		6	
A × A	23	15	47		
A × B	11	9	8	8	4
B × B	1	1			
AB × O	4		5	5	
AB × A	6		10	1	10

CASE REPORTS

The following cases from my own experience are cited because they illustrate some important uses of blood grouping.

P. died shortly after being struck by an automobile. No witness immediately available. The discovery, about a week later, of dried blood some fifty feet from the site of the accident led to the suspicion that he had been attacked near the rocks and then dragged into the road. The rocks and from the clothing worn by the victim on the day of his death were found to be Group B. Witnesses appeared later who testified that they had moved the victim to the place where the blood was found *after* the accident. Although no new facts were obtained by blood grouping in this case, it does illustrate the feasibility of correctly grouping dried blood even when exposed to the elements.

A middle aged man, was accused of sodomy by an eight year old girl. He had been tried for similar offenses at least five times and had served

His technique had always been the same: to lure his victim to a place where he could take her a nickel, assault her, and take back his nickel. Spots on

the child's bloomers contained spermatozoa and the agglutinogens were of Group A. A Wassermann test was ordered on the man for the protection of the child, thus affording the opportunity of determining that his blood group was A. Though insufficient in itself, this finding, in conjunction with the other evidence presented at the trial, was sufficient to bring a verdict of guilty and a prison sentence.

Case 3. J. N., a colored man of Greensburg, Pennsylvania, was arrested on a charge of raping a seven year old white girl. The girl was positive in her identification. Spots of semen on her clothing were identified, and the agglutinogens in this case found to be group B. A specimen of blood from the accused man proved to be of group A, and on this evidence alone the man was released. Subsequent developments justified this decision.



FIG. 1. THE ACCUSED

*Case 4.** This case is presented in some detail, since it appears to be the first of its kind in this country and thus established a much needed precedent.

On April 23, 1931, there came to the laboratory of the Uniontown Hospital a young man (fig. 1) of Italian parentage with an unmarried girl seventeen years of age and her infant son, four months old. The young man's uncle had persuaded his nephew and the girl to have their bloods tested to determine the

* June Sessions 1931, Fayette County, No. 140.

In cross examination the District Attorney entered into a vigorous attack on the "so-called blood test," and was greatly disconcerted by some of the answers made to his questions:

- q. "And is it not also true that a good many pathologists are of the opinion that the parentage of a child cannot be determined by a blood test?"
- a. "They all agree on that subject."
- q. "They all agree?"
- a. "All agree."
- q. "That they can be determined?"
- a. "That they cannot."
- q. "That they cannot be?"
- a. "That is correct."
- q. "Then of what value is the blood test to determine parentage?"
- a. "None."

It was not until later that he realized that the purpose of the testimony was not to prove paternity, but to prove non-paternity.

Many quotations from the Journal of the American Medical Association were placed in evidence by the District Attorney in an attempt to prove that the medical profession had no faith in the value of blood grouping for this type of case. His trump card was a quotation from volume 94, page 1089, April 5, 1930, which is an inquiry addressed to the editor:

I would like some information concerning the present status for medical purposes of all paternity tests by blood grouping; I especially desire to know whether one or both alleged parents can be definitely ruled out if the child in question does not follow in the blood group of one or the other?

ANSWER: The answer to the question as stated is, No. Neither alleged parent can be ruled out simply because the child does not fall in the group of one or the other. For example, parents of groups A and B may have children of all four groups. The child of group O, for instance, could have one parent of group A and the other of group B. This would also be true of a child of group AB. Again a child of group O could have both parents in group A or group B. A child of group B could have one parent in group A, the other in AB. In none of these cases does the child belong to the group of either parent.

Although the above quotation had no direct bearing on this case, the jury failed to understand the significance of the evidence offered, and apparently accepted it as proof of the incompetence of the medical testimony.

In order to prove that blood grouping was in the "experimental" stage and purely theoretical the District Attorney cited the conflicting theories of von Dungern and Hirschfeld with that of Bernstein, and the testimony failed to impress him or the jury that theory and fact were entirely different propositions.

When the judge finally admitted the medical testimony, it was clearly stated that a mother of group A could not have a group B child from a group O man, and that the defendant was not the father.

After the conclusion of the medical testimony the District Attorney exhibited the infant to the members of the jury, calling their attention to the facial resemblance and other points of similarity to the defendant, and said,

The old fashioned evidence produced by eye-witnesses and which has been good enough for these courts from the time they started should not be dashed aside for this new fangled theory yet in its experimental stage.

During the final argument to the jury the Assistant District Attorney made the following statement, with appropriate gestures:

Possible groups of children; if he were certain at the time he made this chart out (referring to table 1), why did he put possible groups of children. In other words, here is what it is: If the parents are O and O, the possible group of the children would be O; if they were O and A, the possible group would be O and A—a possibility. Where is there any certainty about that? Does that exclude this young man? Possibly! The first time I heard about this grouping business was out in Chicago where two children got mixed in the hospital. Well, they grouped and grouped, and as far as I know they are grouping yet, and they are farther away from it than when they started. That is how old and tried this grouping of blood is to determine non-parentage. The mothers finally decided: This is my baby, and the other mother said, This is my baby, and they all went home happy and forgot all about this grouping business.

For the sake of appearances the jury "deliberated" a total of ten minutes and returned a verdict of guilty. When later questioned, one of the more intelligent jurors stated the reason for the verdict as follows:

Of course, we didn't pay any attention to your testimony, and, furthermore, the baby is the image of his grandfather (the father (fig. 2) of the defendant, who appeared as a witness).

After the verdict the attorney for the defense made a motion for a new trial.

The opinion of the Judge is given special consideration because it is the result of several months of study by Judge John Morrow⁵ of Uniontown, who presided at the trial, and his recorded statement, November 7, 1931, furnishes the first legal precedent in this country for the recognition of the importance of blood grouping to exclude paternity. After analyzing the evidence and personally delving into the literature, he concluded that the verdict was against the evidence and granted a new trial. However, this trial has never taken place.

Probably the most valid objection to the acceptance of the medical testimony was the fact that we still tolerate three different methods of designating the blood groups. I agree with the District Attorney that this state of affairs gives the impression of lack of agreement among the members of the medical pro-

fession. Certainly there would be less confusion if the two contradictory methods of designating blood groups by numbers were replaced by the new Landsteiner classification. This classification has international scope and further is the only system which clearly indicates the genetic relationship of the



FIG. 2. THE FATHER OF THE ACCUSED

groups. I disagree with Kennedy,³ that "whichever classification is most prevalent would, to my mind, be the logical one for adoption."

Almost three years after the trial, unexpected characteristics began to develop in the boy whose parentage had been so clearly decided by an intelligent jury.

With great difficulty, contact with the family was made and permission to take a picture of the mother and baby was given. This picture suggests heredity which is not purely Caucasian and the mother's honesty in designating the woodpile as the locale of her amorous adventures is made painfully evident by the photograph of the child. (Fig. 3.)



FIG. 3. THE MOTHER AND CHILD

This photograph was taken three years after the trial

CONCLUSIONS

1. The importance of blood grouping to determine non-paternity is evident when we consider that about half of the men accused in paternity cases are not the true fathers.

2. The laws governing the heredity of blood groups are well known and properly controlled observations of the blood group of the true parents and their children, fail to reveal exceptions to these laws. Personal observations of the blood groups of 240 "certified" parents and their 259 children are quoted.

3. The confusion in the lay mind arising from the existence of three methods of designating the blood groups, should be ended by having this society officially adopt the Landsteiner classification. This classification is urged, first because it gives

proper credit to the original discoverer of the blood groups; second because it is international in scope; third, it eliminates two contradictory methods of designating the groups by numbers; and, finally, the Landsteiner terminology indicates the genetic relationships of the blood groups.

4. Details of a court trial are given, showing the inability of the prosecuting attorneys to understand the scientific basis and value of blood grouping, and a final jurors' verdict based on the "good old fashioned evidence of eye witnesses and not upon this new fangled theory" is given.

5. I believe that no case of fornication and bastardy should appear in the courts of our country unless blood grouping, performed by properly qualified persons, fails to exclude paternity on the part of the accused. Also refusal of the mother to permit blood grouping should be given due consideration by the legal profession.

6. The opinion of Judge John Morrow furnishes a much needed precedent in recognizing the value of blood grouping.

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PREPARATION OF NEUROLOGIC MATERIAL FOR HISTOLOGIC STUDY*

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In order to arrive at a correct diagnosis of lesions of the nervous system, pathologists are faced with an overwhelming assortment of special staining methods which are tedious and complicated, and usually require special fixatives. There is much duplication of methods; this has the advantage of allowing the pathologist a wide choice, but has the disadvantage of leading to confusion. Even expert technicians find difficulty in employing many of these staining methods, and much simpler and less complicated and time consuming methods often serve the purpose equally well.

The suggestions on histologic methods which are offered here are not intended to take the place of text books on the subject of neurohistologic technic, or of methods used in neurologic research. They represent, merely, some of the common and comparatively simple methods used in the preparation of neurologic material for histologic study. Each pathologist, of course, develops in his own laboratory a few methods with which he becomes familiar and from which he obtains most information. His technicians also, as the result of frequent repetition, do better with these than with any other methods. The following methods, however, may be used to supplement those which are used routinely in most general histologic laboratories. The list is far from complete, but by the use of the methods given most of the elements in normal and abnormal tissues of the nervous system can be demonstrated.

It will be necessary on occasion to make a diagnosis on fresh neoplastic tissue removed at operation, so that the surgeon can

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decide what, if any, further operative procedure should be done. Staining fresh, frozen sections with polychrome methylene blue, as used by MacCarty and Broders for many years, is eminently satisfactory and is a method that has stood the test of time, although one of several more recent modifications may be adopted.

The proper fixation of tissue removed at operation is very important, and due regard must be paid to the nature of the tissue and to the staining methods which may be necessary to arrive at a correct diagnosis. It is occasionally impossible to foresee these conditions and, therefore, a few pieces as fresh as possible should be placed in Zenker's and in Cajal's solutions and the remainder in a 10 per cent solution of formol. When a neoplasm is present and the entire brain has been removed at necropsy, small portions should be fixed in similar solutions as recommended for tissues removed at operation and even though a neoplasm is not present, it is frequently advisable to remove small portions from various parts of the brain anyway and to place these in the Zenker and Cajal solutions before the brain is fixed in formol. In order to fix the brain in formol, the preferable method is to inject a 10 per cent solution of this mixture into one carotid and into one vertebral artery, or else to suspend the brain in the 10 per cent formol solution by a thread passed between the basilar artery and the pons and held so that the vertex does not rest on the bottom of the container. After the brain has been fixed for a few days, it is best to cut it by coronal sections about 1.5 cm. apart. This permits careful examination and easy reconstruction of the tissue, and also makes the sections suitable for photographing. Small portions can be removed at this time for microscopic preparation and study.

The universal fixative in all pathologic laboratories, of course, is a 10 per cent solution of formol, which has the advantage of preserving tissue almost indefinitely and of permitting the use of the majority of routine staining methods.

Stains for lipoids are sometimes necessary to demonstrate active degeneration of myelin sheaths in the brain, spinal cord, or in peripheral nerves (Method 1). This staining is done after

the tissue is fixed in formol solution and cut in frozen sections. Frozen sections are necessary in the Bielschowsky silver method to demonstrate axis-cylinders, but a modification of this stain can be used on formol-fixed tissue which has been embedded in paraffin. Contrary to the usual teaching, embedding of neurologic material in celloidin is not necessary except for some special and rarely used methods. I have long since ceased to use celloidin as a routine embedding medium and have substituted paraffin. After embedding in paraffin, the routine histologic stain, hematoxylin and eosin, is used. This is satisfactory to demonstrate inflammatory reactions and is the routine stain for all neoplasms of the central nervous system, although one or more special stains are used to confirm the diagnosis. Van Gieson's stain is useful to demonstrate connective tissue, but it is not so satisfactory for this purpose as the Perdrau silver method (Method 2). The impregnation method has been very satisfactory in this laboratory, but there are several other equally good silver impregnation methods for connective tissue (Laidlaw, Foot, and so forth). There are two rapid methods for demonstrating myelin sheaths after formol fixation and paraffin embedding (Loyez and Weil), but they are not as satisfactory as the original Weigert myelin sheath stain or Pal's modification of the original Weigert stain.

Changes occur in nerve cells as the result of trauma, inflammation, injury following toxins, deficiency in diet, poisons, and so forth, and, to see these changes, stains used to demonstrate Nissl's granules must be used. There are many such stains and all are modifications of Nissl's original methylene blue. Two of the most satisfactory of these are toluidine blue stain and thionin stain (Method 3). Many of the stains used to demonstrate Nissl's granules fade more or less rapidly, and the methods require overstaining of microscopic preparations which are then decolorized; it is necessary, therefore, to wash away completely all decolorizing fluid to prevent such fading.

To demonstrate nerve cells and axis-cylinders, silver impregnation methods also are best, and these usually are some modification of Bielschowsky's method. At the clinic a special

modification has been used for the last seven years and has been found to give satisfactory and uniform results. This method reveals changes in the neurofibrils of ganglion cells and degeneration of axis-cylinders, especially changes of the axis-cylinders of peripheral nerve fibers in so-called neuritis and similar conditions. Its chief advantages are that it can be done after formol fixation and paraffin embedding, it is comparatively simple, and, after a little experience, it is reliable and uniform (Method 4).

Some of the most valuable stains available to the neuropathologist are the Mallory stains and some of their modifications, especially the Mallory phosphotungstic acid-hematoxylin and the Mallory-Heidenhain stains for the study of neuroglia, connective tissue, and so forth. The Mallory phosphotungstic acid-hematoxylin stain makes connective tissue red to reddish-purple and glial tissue blue. This stain is particularly valuable in demonstrating neuroglial fibrils which stain blue (Method 5). The Mallory-Heidenhain stain is one of the most valuable stains in the study of peripheral nerves, spinal cord, pituitary gland, and so forth. This stain makes connective tissue sky-blue, myelin sheaths reddish-orange, and axis-cylinders purple. This stain is also very valuable in the study of tumors of the pituitary gland, since it brings out clearly the three types of cells. Mallory's ethyl-violet orange G stain can be used for the same purpose, but is not quite so satisfactory, and the colors not so vivid or distinctive. Tissue can be stained satisfactorily with these stains only when it has been fixed immediately after removal in Zenker's fixing solution for twenty-four hours. After this fixation sections must be treated with Lugol's solution to remove the bichloride of mercury which otherwise would be scattered as crystals diffusely over them.

These extremely valuable stains could not be used after tissue was fixed in formol solution, and refixing in Zenker's solution was not satisfactory; as a consequence, many attempts have been made to adapt formol-fixed material to Mallory's stains. A method has been used for some years at the clinic which has proved highly satisfactory for the adaptation of formol-fixed

material to Mallory's stains (Method 6). This method has continued to give good results since a report of it was published in 1931, and it will be found particularly useful for the demonstration of fibrin, neuroglia, fibroglia, and myoglia fibrils. It also brings out with great sharpness and faithfulness of detail the structure in mitotic figures, including those of spindles and centrosomes. Formol-fixed tissue may be deformolized by placing it in ammonia water for one to several days depending on the time the tissue had been in formol solution. It is then refixed in Zenker's solution for twenty-four hours or, better still, in Weigert's mordant I (Method 7) for forty-eight hours, and then transferred to Weigert's mordant II (Method 8) for four days; it is then embedded in paraffin. This tissue is then stained after the manner of Mallory or some of the modifications of his method.

An advantage is using Weigert's mordants after tissue has been fixed in formol solution is that the Weigert myelin-sheath stain can also be used. There are numerous modifications of this stain, but at the clinic the original Weigert stain has continued to be used because it has been found more uniform and constant than any of the modifications. This stain renders myelin bluish-black and all other tissue tan, except erythrocytes which are black (Method 9).

One of the traditional stains with which to demonstrate active degeneration in nerve tissue is the Marchi stain, but it has not been used at the clinic for some years because of the precipitation of crude osmium on tissue, which produced artefacts and led to misinterpretation, and also because of the difficulty of obtaining uniform penetration of this stain into the tissues. A preferable method of demonstrating such degeneration has been found to be the use of some of the fat stains, preferably Scharlach R, on frozen sections of formol-fixed material.

In recent years there has been a great increase in knowledge of the various types of glial cells, and this has been encouraged by better staining methods, particularly those of gold and silver impregnation. The larger glial cells, the astrocytes, which together with the blood vessels form the frame-work of the nervous system, are best demonstrated by Cajal's gold chloride

and sublimate method. This method is uniform, comparatively simple, and the results are excellent. It reveals cell bodies with all their ramifications and vascular attachments as well as intracellular fibrils. The original Cajal's method has not been improved on (Method 10). The results are unsatisfactory if the tissue has been fixed in commercial formol solution, so that a special fixative (the Cajal fixative) is necessary. The tissue is placed in this Cajal fixative for from four days to two weeks, and sections are cut frozen. The gold chloride solution must be made fresh each time it is to be used. If the solutions are properly made, and the tissue fixed in formol-bromine mixture, the results are uniform and excellent. The other two types of glial cells are not impregnated by the gold chloride method, but require impregnation by silver carbonate solution. These cells are (1) the oligodendroglia cells which are found in the cortex of the brain as satellite cells, and so forth, and are very numerous in white matter and (2) the microglia which represent the reticulo-endothelial cells of the brain and are the phagocytic cells of the central nervous system. These latter cells have numerous names: rod cells, gitter cells, compound granular corpuscles, scavenger cells, and so forth. Both these types of glial cells can be impregnated by the del Rio Hortega silver carbonate method after having been fixed in formol-bromine solution and after frozen sections have been made from the same block from which sections were cut to be stained with Cajal's gold chloride and sublimate method. There are several modifications of the Hortega method available, and these are slightly simpler and more uniform than the original method. At times, beautiful results can be obtained, but the chief objection to this method is the lack of uniformity in results; further work needs to be done both to simplify the procedure and to obtain more constant impregnation of cells. However, if care is taken in preparing the silver carbonate solution, so that all the precipitate is just dissolved and yet the solution is not too alkaline, the results will be satisfactory (Method 11).

There are numerous other staining methods that can be used in addition to these or to replace some of them, but only those

stains have been described which have been found at the clinic to be most suitable and reliable. The foregoing staining methods, however, are not always used for the study of all tissues, and only those stains are used which will be of help in demonstrating special cells or changes in definite disease processes; occasionally, therefore, additional methods are necessary. Several examples of stains used to demonstrate definite lesions are given so that they may be of help in indicating the uses of these stains.

In acute inflammatory lesions, such general stains as are used to demonstrate inflammation and its reactions in other parts of the body are usually sufficient. In chronic inflammation of the central nervous system, such as parenchymatous syphilis (general paresis), hematoxylin and eosin stain reveals the inflammatory and mesodermal reactions, but to demonstrate the changes in nerve cells and glial reactions, special methods are necessary. The thionin or toluidine blue stain demonstrates the degeneration of ganglion cells, also the fact that the normal architecture of the cortex is upset and that nerve cells are no longer in orderly arrangement; the modified Bielschowsky silver method will also demonstrate the disarrangement of ganglion cells. In order to appreciate the increased numbers of glial cells (astrocytes) Cajal's gold chloride and sublimate method is necessary. This method reveals marked gliosis in the cortex of the frontal lobes, whereas Hortega's silver carbonate method is necessary to demonstrate the increase in microglia, which are forerunners of most of the scavenger cells of the cerebrum. Any iron stain will reveal the well-known fact that, in paresis, there is a considerable deposit of iron in the cortex. If it is desired, Pappenheim's stain for plasma cells will indicate that many of the inflammatory cells in the perivascular spaces are plasma cells.

In recent years, there has been a marked increase in our understanding of that group of tumors under the heading "gliomas," and neurologists and neurosurgeons request more specific information as to type because on this further classification depends the extent of the operative procedure and of the radium or roentgen-ray treatment, and the ultimate prognosis. In the identification of various gliomas, special staining methods are valuable

and are necessary to substantiate the opinion arrived at by using the general stains. At the clinic, hematoxylin and eosin is the routine stain for all tumors. When possible, small portions of the tumor are fixed in Zenker's solution and in formol-bromine mixture. Cajal's gold chloride and sublimate method is useful since it demonstrates the astrocytes of astrocytomas, the astroblasts of astroblastomas, and also the several types of spongioblasts; this method reveals the dominant-cell type in some of the mixed types of gliomas. Mallory's phosphotungstic acid-hematoxylin stain demonstrates neuroglial fibrils in astrocytomas and distinguishes them from other types of gliomas; it is also helpful in demonstrating blood vessels and such changes as the proliferation of lining endothelium of the dilated, thin-walled blood vessels of rapidly growing gliomas. This stain is also valuable in distinguishing connective tissue from neoplastic tissue in meningiomas. The Perdrau silver impregnation method for connective tissue gives a beautiful outline of the blood vessels and blood spaces in hemangio-endotheliomas and, when done on frozen sections in conjunction with a fat stain, it shows the relation of fat-containing endothelial cells to the blood spaces of these tumors. Hortega's silver carbonate method for oligodendroglia is useful, at times, in demonstrating these cells in oligodendrogliomas, but it is unsatisfactory as a general rule and not very reliable.

In some chronic degenerative diseases of the central nervous system, such as multiple sclerosis, combined degeneration (usually associated with pernicious anemia), tabes dorsalis, amyotrophic lateral sclerosis, and so forth, special staining methods are necessary to reveal the specific changes that are diagnostic. In tabes dorsalis, the degenerative condition of the posterior columns can only be shown well with a myelin sheath stain. When this stain is used, the anterior and most of the lateral columns will be blue-black, whereas posterior columns, peripheries of the lateral columns, and gray matter, will be tan. Mallory's phosphotungstic acid stain, which gives excellent results after the Weigert's mordants have been used, and can thus be used on sections cut from the same blocks and at the same

time that the sections for the Weigert stain were cut, stains blue the posterior columns and peripheries of the lateral columns, indicating an excess of glial cells and of neuroglial fibrils. Scar tissue of the central nervous system is neuroglia and, in *tabes dorsalis*, multiple sclerosis, and so forth, represents replacement of degenerated myelin. The modified Bielschowsky impregnation method on longitudinal sections of the spinal cord reveals that, in *tabes dorsalis*, axis-cylinders have disappeared from the areas in which myelin has been destroyed, whereas in multiple sclerosis, they are preserved except in the oldest and most advanced lesions. This method gives excellent results when done on frozen sections in conjunction with a fat stain in cases of recent injury or active degeneration where the myelin has degenerated into neutral fat and axis-cylinders are actually degenerating. Many other methods may be used for diseases of the spinal cord, but the foregoing usually are sufficient except in cases of meningitis, myelitis, or poliomyelitis, where general stains show the inflammatory reaction and toluidine blue or thionin stains reveal the degeneration of large anterior horn cells. Cajal's and Hortega's methods are not so valuable in studies of the spinal cord as in those of the brain. The same staining methods, however, apply to tumors of the spinal cord as to those of the brain. There are many diseases involving the peripheral nerves, but by far the most common of these is so-called peripheral neuritis with its numerous clinical subdivisions. True inflammation of peripheral nerves is extremely rare, whereas degenerative conditions are much more common. The changes in degenerative lesions of peripheral nerves can readily be demonstrated. The modified Bielschowsky silver impregnation method reveals the swelling, beading, and fragmentation of the axis-cylinders, whereas although Weigert's myelin-sheath stain shows changes of the myelin sheaths the Mallory-Heidenhain stain has been found at the clinic to be a better one. This stain can be applied best after the use of Weigert's mordants, or after Zenker's solution and Weigert's mordant II, and it shows myelin reddish-orange, connective tissue blue, and axis-cylinders pale-purple. By using this stain, the blood vessels are seen easily and it is on

the basis of vascular changes that most of the degenerative conditions of the peripheral nerves depend. The Mallory-Heidenhain stain is better for studying lesions of peripheral nerves than those of the central nervous system. The hematoxylin and eosin stain will also show inflammatory changes if any are present, and van Gieson's stain will reveal any increase of connective tissue or increase of the thickness of the walls of blood vessels with attendant narrowing of the lumens. On this basis most so-called neuritis occurs, and it should more properly be called ischemic neuritis.

SUMMARY

Since many text books have been devoted to neurologic technic, it is obvious that the above methods are very incomplete, but by their use, most lesions of the nervous system can be detected and a correct diagnosis reached. The methods presented in this paper are not the only ones, or necessarily the best ones, but I have found them useful, for several years. Most of them could be replaced by others equally good and satisfactory. It is not the object of this small contribution to replace the text books; the purpose has been rather to simplify the methods and to make possible the study of neurologic tissue in any laboratory without the necessity of elaborate equipment or of very complicated staining methods. With a little experience these methods will be found to be quite reliable, and reactions in the nervous system to inflammation and disease will be fairly easy to demonstrate and to understand.

APPENDIX

METHOD 1

Fat staining method

1. Fix tissue in formol solution, freeze and section, 10 to 20 microns.
2. Wash sections in 70 per cent alcohol for one minute.
3. Stain for five minutes in fat stain, made as follows:
 - Sharlach R, 1 gm. (sudan IV may also be used)
 - Absolute alcohol, 70 cc.
 - Distilled water, 30 cc.

(Heat this mixture just a little, and shake while heating. Then add 100 cc. acetone and put in a 37°C. oven overnight. Always filter stain before using.)

4. Wash in 70 per cent alcohol quickly.
5. Stain in hematoxylin (Harris) for five minutes.
6. Wash in tap water.
7. Dip in (1 per cent) acid (70 per cent) alcohol about two seconds.
8. Wash in tap water ten minutes or longer.
9. Wash in lithium carbonate (saturated aqueous solution) about two seconds.
10. Wash in tap water and mount with glycerine.

METHOD 2

Perdrau impregnation method

1. Paraffin sections run down to water in usual way.
2. Place for twenty to thirty minutes in 0.25 per cent solution of potassium permanganate.
3. Wash in water.
4. Place in solution of equal parts of 1 per cent oxalic acid and 1 per cent potassium sulphite, or 5 per cent oxalic acid, until color is gone.
5. Wash for a half to one hour in tap water until all acid is washed out (limit the length of time in case the sections come loose).
6. Place sections in a 20 per cent solution of silver nitrate for half an hour.
7. Wash quickly in double-distilled water.
8. Place sections in the following solution:
Silver nitrate (20 per cent) solution, 5 cc.
Sodium hydroxide, 6 drops.
(Add ammonium hydrate drop by drop until the precipitate is almost destroyed. Add distilled water up to 50 cc. and filter. Leave sections in this solution for half an hour.)
9. Wash in double-distilled water.
10. Fix in 10 per cent formol solution (neutral).
11. Wash in double distilled water.
12. Tone in gold chloride solution 1-500.
13. Fix in sodium hyposulphite.
14. Wash in tap water, dehydrate, clear, and mount.

METHOD 3

Toluidine blue, or thionin, staining method

1. Run sections down to water.
2. Place sections in toluidine blue or thionin, stain for fifteen to twenty minutes (heat stain before using for thirty minutes):
1:500 solution = 1 gm. toluidine blue or thionin to 500 cc. water.
3. Wash in two changes of 95 per cent alcohol (color comes out rapidly).

4. Place in absolute alcohol and watch under microscope until differentiation is complete.
5. Clear in xylol, thoroughly, and mount with Canada balsam.

METHOD 4

Modified Bielschowsky impregnation method

1. Fix tissue in solution of formol, 10 per cent.
2. Embed section in paraffin and cut at 10 microns.
3. Deparaffinize.
4. Wash three times in double-distilled water.
5. Place in 20 per cent solution of silver nitrate for one hour.
6. Wash quickly (twice) in double-distilled water.
7. Transfer sections into ammoniated solution of silver nitrate for five minutes:
To 40 cc. of 20 per cent silver nitrate solution add ammonium hydrate drop by drop until precipitate is dissolved. An excess of ammonium hydrate is noxious. Make a fresh solution each time it is to be used. Filter before using.
8. Wash quickly in double-distilled water.
9. Transfer sections into 10 per cent solution of formol (neutral) for one minute.
10. Wash, tone in gold chloride (brown) solution (a), fix in sodium hyposulphite solution (b), one or two minutes, clear (c), and mount (d):
 - (a) Gold chloride, 1 gm.
Water, 500 cc. (can be kept as a stock solution)
 - (b) Sodium hyposulphite, 5 gm.
Water, 100 cc.
 - (c) 95 per cent alcohol
Acetone
Carbo-xylol
Xylol
 - (d) With Canada balsam

METHOD 5

Mallory-Heidenhain's staining method

1. As a fixative, use formol or Zenker's solution, or Weigert's mordants. Cut paraffin sections at 5 microns.
2. Stain forty minutes in:
Azo-carmin (Grubler's), 1 gm.
Water, 100 cc.
(Heat, cool, and filter at room temperature and add 1 cc. of glacial acetic acid before using.)
3. Wash in water.

4. Differentiate in aniline alcohol (watch under microscope) until nuclei are red and cytoplasm is pale-pink. This step requires from three to ten minutes depending on thickness of sections.
5. Remove aniline alcohol with acid alcohol (1 per cent) a half to one minute.
6. Wash quickly in water.
7. Place in 5 per cent phosphotungstic acid for three hours.
8. Wash quickly in water.
9. Stain one-fourth to one-half hour in Mallory's aniline blue made as follows:
Aniline blue, 0.5 gm.
Orange G, 2 gm.
Water, 100 cc.
Acetic acid, 8 cc.
(Boil, cool, filter, and thin with equal parts of water before using.)
10. Wash quickly in water.
11. Differentiate in absolute alcohol.
12. Clear in carbo-xylol and xylol.
13. Mount with Canada balsam.

METHOD 6

Mallory's phosphotungstic acid-hematoxylin staining method

1. Fix in Zenker's solution for twenty-four hours.
2. Place in running water for twenty-four hours.
3. Embed in paraffin.
4. Section and deparaffinize.
5. Place in potassium permanganate for from five to twenty minutes (make a fresh 0.25 per cent aqueous solution each time).
6. Wash well in water.
7. Place in oxalic acid (5 per cent aqueous solution) for from five to ten minutes.
8. Wash thoroughly in several changes of water.
9. Stain with Mallory's phosphotungstic acid-hematoxylin stain three to twelve hours. This stain is made as follows:
Ammonia hematin, 0.1 gm.
Water, 100 cc.
Phosphotungstic acid crystals (Merek), 2 gm.
(Dissolve the ammonia hematin in a little water, with the aid of heat, and add it when cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 cc. of a 0.25 per cent aqueous solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously. Hematoxylin may be used instead of ammonia hematin, but requires 10 cc. of the permanganate solution to ripen it).
10. Place sections in 95 per cent alcohol, then in absolute alcohol, and clear in xylol.
11. Mount with Canada balsam.

METHOD 7

Weigert's mordant I

Potassium bichromate, 5 gm.

Fluorochrome, 2 gm.

Water, 100 cc.

(Fix tissue in mordant I for from four to five days, for small and thin sections, two days.)

METHOD 8

Weigert's mordant II

Acetate of copper, 5 gm.

Fluorochrome, 2.5 gm.

Acetic acid (36 per cent), 5 cc.

Water, 100 cc.

Formol, 10 cc.

(Fix tissue in mordant II for from twenty-four to forty-eight hours.)

METHOD 9

Weigert's myelin-sheath staining method

1. Wash tissue, which has been fixed in 10 per cent solution of formol, in water for short time.
2. Fix in primary mordant four to five days (for small and thin sections, two days).
3. Wash in water one hour.
4. Fix in secondary mordant twenty-four to forty-eight hours.
5. Wash well in water one to two hours.
6. Embed sections and deparaffinize.
7. Stain two hours in a solution made as follows:
 - 10 cc. of a 10 per cent solution of hematoxylin dissolved in absolute alcohol
 - Saturated solution of lithium carbonate, few drops
 - Distilled water, 90 cc.
 - (Make this solution fresh each time)
8. Wash in tap water.
9. To differentiate, place in a solution of:
 - Borax, 2 gm.
 - Potassium ferricyanide, 2.5 gm.
 - Water, 100 cc.
10. Wash thoroughly in running water.
11. Dehydrate in 80 per cent and in 96 per cent alcohol.
12. Clear in acetone, carbo-xylol, and xylol.

13. Mount with Canada balsam.

(A stock solution of 10 per cent solution of hematoxylin in absolute alcohol should be kept on hand. At least ten days' exposure to sunlight is required to ripen this solution. Combine it with a few drops of saturated aqueous solution of lithium carbonate at the time of using.)

METHOD 10

Cajal's gold chloride and sublimate method

1. Place small portions of fresh tissue in the following solution for two to ten days (the tissue may have previously been fixed in formol solution a very short time):

Formol (neutral), 15 cc.

Ammonium bromide, 1.5 to 2 gm.

Distilled water, 85 cc.

(Tissue should not remain more than ten days in this solution, seven days is best)

2. Cut sections on freezing microtome (20 to 25 microns) and place in 6 per cent neutral formol solution.

3. Wash a few moments in distilled water.

4. Place in following solution for six to ten hours and keep in the dark:

Gold chloride (1 per cent), 10 cc.

Mercuric chloride, 0.5 gm. (10 cc. of a 5 per cent solution)

Distilled water, 60 cc.

(Dissolve the mercuric chloride by heat in nearly all water called for in the formula, filter, and add to the gold chloride. The best temperature is 18° to 20°C. (65° to 68°F.) At this temperature, four to six hours is the best.

If temperature is 14° to 17°C., the time should be much longer. If temperature is higher, the time should only be two to three hours.)

5. After four or more hours, when sections are intense purple, fix for from six to ten minutes in the following solution:

Sodium hyposulphite, 5 cc.

Alcohol (95 per cent), 30 cc.

Distilled water, 70 cc.

Saturated solution bisulphite of soda, 5 cc.

6. Wash in 50 per cent alcohol.

7. Place on slide, blot carefully, then dehydrate on slide with 70 per cent, and 95 per cent, and absolute alcohol; then clear in xylol.

8. Mount with Canada balsam.

(Blot carefully after each of above steps. Oil of origanium may be used in place of absolute alcohol.)

METHOD 11

Hortega silver carbonate impregnation method for oligodendroglia and microglia

1. Fix tissue in formol-ammonium bromide solution (same as for Cajal's stain) and cut frozen sections.
2. Wash in two dishes of distilled water, the first containing 10 drops of ammonia.
3. Stain in undiluted silver carbonate for from one minute to one and a half hours. Del Rio Hortega's undiluted ammonical silver carbonate is prepared as follows:
Solution of silver nitrate (Merck), 10 per cent, 5 cc.
Solution of sodium carbonate (pure), 5 per cent, 20 cc.
Ammonium hydroxide, sufficient to dissolve precipitate
(The ammonium hydroxide should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while.) Finally, filter the solution and place in a dark bottle, where it will keep for long periods.
4. Wash rapidly in 60 per cent alcohol (made by using absolute alcohol). The section should be carried through with a small angulated glass rod so as to allow all of it to be washed equally without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.
5. Reduce by passing sections directly in 1 per cent formol solution.
6. Wash in distilled water.
7. Tone by placing sections in a gold chloride bath ten to fifteen minutes until they become purple-gray.
Gold chloride (yellow), 1 gm.
Distilled water, 500 cc.
8. Fix in a 5 per cent solution of sodium hyposulphite for half a minute or more until sections are flexible.
9. Wash in water.
10. Dehydrate in dishes of graded alcohol and clear in carbo-xylol and xylol.
11. Mount with Canada balsam.

FURTHER STUDIES IN ANTIGEN EMULSION PREPARATION FOR THE BALL TEST FOR SYPHILIS*

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Several years ago a ball test for syphilis was described.² The results with this test are very easy to read accurately with the naked eye. The negative test shows a uniformly turbid fluid. A strongly positive test on the other hand shows a thoroughly clear fluid and suspended in its upper portion a large opaque white ball. A positive test shows a few large white clumps in the upper half of a slightly turbid fluid and a doubtful test shows numerous small white clumps suspended in turbid fluid. The phenomenon of ball formation in this test is apparently dependent upon the interlocking of numerous small clumps of needle like particles which dovetail into each other upon centrifugation. The ball test applied in two slightly different ways gives results of about equal sensitivity with the two microscopic slide precipitation tests (1, for the diagnosis and 2, for the exclusion of syphilis) and serves as an excellent check for the slide tests.

The antigen emulsion for the ball test as described is prepared by mixing salt solution and cholesterinized antigen, the resultant combined antigen cholesterol particles although of desirable shape (long thin needles) are satisfactory for use for only a few to several hours after preparation following which time the emulsion breaks down, the particles become granular and the globules of antigen only present in the emulsion clump together.

As described previously,² emulsions generally employed in precipitation tests for syphilis (including the ball test mentioned above) are made by mixing salt solution with cholesterinized antigen. The resultant aggregates are composed of antigen

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lipid, cholesterin and adventitious substances more or less uniformly distributed throughout the particles. In addition there are a number of globular particles of antigen only. The size and shape of the combined antigen cholesterin particles and particles of antigen only are determined especially by the quantitative relationship of lipid and water, by the temperature of the ingredients and by the speed at which the mixture is made. In emulsions chemically identical for instance the particles vary from less than 1μ in diameter when very warm ingredients are quickly mixed to large needle like particles 60μ or more in length when very cold ingredients are slowly mixed. The particles furthermore undergo continuous change in size and shape and in a few minutes to several hours are unsatisfactory for use in tests for syphilis.

In contrast to such emulsions, those employed in the microscopic slide precipitation tests for syphilis are made by first precipitating the cholesterin crystals in water and subsequently coating them with antigen lipid. Cholesterin plates precipitated from alcoholic solution by a small or large amount of warm or cold water or salt solution, by slow or by rapid mixture vary but little in size and shape. Upon this fact depends the greater uniformity in particle type and of sensitivity of slide test emulsions in routine practice. Furthermore the particles maintain their size and shape for over a week and the emulsions containing them are thoroughly satisfactory for use for at least 48 hours after preparation.

It is apparent therefore that the ball test for syphilis previously reported can be improved by preparing stable needle like particles to hold antigen on their surfaces. This has been found possible by two methods:

(1) Addition of about equal parts of Squibb's anesthetic ether to 1 per cent alcoholic solution of cholesterin and the addition of cold water to this combined solution after it has been chilled.

(2) Addition of 4 parts chilled 0.5 per cent cholesterin solution in glacial acetic acid to 1.75 parts chilled 60 per cent alcohol.

The needles prepared by the ether method are rather fragile and rather sensitive to an excess of electrolytes. The needles

prepared by the acetic acid method are most satisfactory in this regard and furthermore after they are removed from the acetic acid and alcohol solution and properly neutralized with 7 per cent sodium hydroxide they serve excellently in holding antigen on their surface. Emulsions containing these antigen coated needles in proper proportions with serum (about 1 part emulsion, 5 parts serum) have given results similar to the ball test previously described and have been found satisfactory for use for at least 24 hours after preparation. It is hoped before long to have all the details of emulsion preparation and test performance worked out.

In addition to these studies with antigen coated cholesterin needles some experiments have been made using globules of antigen only as in the Hecht¹ ball test. In that test antigen globules are precipitated from alcoholic solution by about a half part of salt solution and after a ten minute period of ripening another half part of salt solution is added. At this time as after the first step the antigen globules are in small clumps which are dispersed by the patient's serum whether positive or negative. In addition to the globules, which vary greatly in size from less than 1μ to about 25μ there are many elongated tongue shaped particles and these readily dovetail into each other in positive tests.

Since the size and shape of particles of antigen only in water or salt solution are influenced by so many factors the possibility of preparing uniformly sensitive ball test emulsions with antigen alone is not as great as with antigen coated needles of uniform size and shape.

SUMMARY

In the ball test for syphilis described several years ago the emulsion particles of combined antigen and cholesterin are unstable and satisfactory for use for a few to several hours only.

Further studies have demonstrated the possibility of preparing emulsions with stable antigen coated cholesterin needle like crystals that are satisfactory for use in the ball test for at least 24 hours.

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CYANIDE POISONING*

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Hydrocyanic acid as a poison was said to have been used by the ancients, usually in the form of an infusion of peach stones or seeds of other fruits in which hydrocyanic acid was liberated. However, accurate records on this subject are not available. One of the earliest authentically recorded cases occurred in Germany in 1781.^{4,5} For a time in the early and middle parts of the last century the cyanides were said to have been a popular method for committing murder and suicide in this country, but here again accurate statistics were not kept.

In recent years suicidal, accidental and industrial cyanide poisonings have occurred with increasing frequency. Homicidal attempts have been rare. Deliberate poisonings have usually been produced by mixing potassium or sodium cyanide in food, drink or medicines. Accidental poisonings result chiefly from fumigation, mistaking a container of cyanide for a medicine, in chemical laboratories and in certain industrial occupations. The industrial type results from the escaping vapors in certain chemical processes, notably the silver, gold, celluloid and polishing industry. Domestic animals (goats, sheep, cows) and even man are found now and then either dead or suffering from symptoms of cyanide poisoning as a result of the ingestion of fruits sprayed with an insecticide containing cyanide or from plants that are relatively rich in the glucosid amygdalin (peach leaves, cherry, laurel, et cetera). The latter is broken down into sugar, oil of bitter almonds and hydrocyanic acid in the presence of water

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and emulsin. It is of considerable practical significance that hydrocyanic acid is a poison for all members of the animal kingdom³ (rodents, flies, bugs, et cetera). Its use as a lethal gas in the late war was not as successful as were some of the other gases. Its use as a method for legal execution has not gained much popularity.

McKelway⁷ records an interesting case in which a hairdresser, aged 38 years, attempted to remove "silver discoloring" of hair dye from her hands by rubbing them with potassium cyanide. At first the patient developed symptoms of vertigo, nausea, et cetera (probably caused by inhalation of cyanide) which disappeared only to recur about one and one-half hours later in a much more aggravated form from which the patient, however, recovered. Absorption of cyanide through the skin was thought to have taken place, though it was not noted whether she had washed her hands free of the salt after her first attack. Experimental absorption of hydrogen cyanide through the skin has been proved and Drinker¹ has observed it in human beings. Accidental poisonings through the injured skin have been frequent and recently mention has been made of poisoning developing from silverware used at table which had been cleaned with a polish containing cyanide, the utensils not having been properly washed.

SYMPTOMS

The symptoms of acute poisoning proceed with almost lightning-like rapidity. Within two to five minutes after ingestion of the poison, the individual collapses, frequently with a loud scream (death scream); there are a few short dyspneic inspirations followed by prolonged expirations; trismus and perhaps tetanus may be observed; bloody, frothy saliva and vomiting may occur. In cases, especially as a result of admixture with foods, where absorption is not quite as rapid, Lewin⁶ divides the symptoms of cyanide poisoning into three stages, as follows:

(1) *Dyspneic stage.* Sense of constriction in the throat, oppression, fear, staggering walk, nausea or vomiting, headache, vertigo, weak pulse, prolonged respirations with short inspirations and prolonged expirations, and the intervals between breaths becoming longer and longer.

(2) *Stage of convulsions.* The patient falls, the pupils are dilated, and the skin is covered with a cold clammy perspiration. The pulse becomes more rapid, convulsions, opisthotonus, trismus and involuntary micturition ensue.

(3) *Stage of asphyxia.* Respirations become irregular and prolonged, the heart irregular and slow, the body temperature drops, cyanosis of face and extremities is noted, the patient sinks into a coma and frothy, often blood-tinged saliva flows from the mouth. Death usually follows within a half hour.

In some patients who have recovered from poisoning, complaints of weakness, fatigue, insomnia and cardiac distress continuing for months are occasionally encountered.

The symptoms of chronic cyanide poisoning vary considerably with each person, dependent apparently on individual idiosyncrasies and susceptibilities. Some individuals by merely entering a place in which cyanide is handled, quickly develop symptoms of nausea, headache, giddiness and scratching or irritability of the throat. Others may work in an atmosphere containing cyanide in comparative comfort or develop symptoms only after prolonged exposure or contact. In a well developed case of chronic poisoning, the patient usually complains of lassitude, easy fatigue, attacks of nausea and vertigo, occasional vomiting, headache, insomnia, cardiac discomfort, anemia, skin eruptions, and occasionally ocular and auditory disturbances. The vagueness of the symptoms makes a diagnosis at times exceedingly difficult, unless the patient's occupation yields a clue. In the combustion of tobacco during smoking appreciable amounts of hydrogen cyanide vapors are formed, though nothing at present is definitely known as to its effect in intensive smokers.

The action of cyanide in the body is described as interfering with the "internal" cell respiration by paralysis of the oxydase ferments. Thus oxidation and reduction cannot continue in the cells. Hanzlik and Richardson³ experimentally and Geiger² clinically have used a 1 per cent solution of methylene blue (containing 1.8 per cent sodium sulphate) in cases of cyanide poisoning with good results. The basis of the action of methylene blue is to convert the hemoglobin into methemoglobin which then

combines with the cyanide forming cyamethemoglobin, which is innocuous.

· NECROPSY

Postmortem examination of an acutely poisoned subject frequently shows bright red spots in the skin and cyanosis of the face, neck and extremities. On opening the body the odor of hydrogen cyanide is distinct and almost invariably noticeable. The blood, depending on the number of hours postmortem, is generally quite dark, remains fluid and engorges the veins and right heart. Frequently the mucous membrane of the lips, mouth and esophagus, especially if the poison is taken without an admixture of food or drink, is swollen and shows deep reddish discoloration; rarely corrosion may occur. The stomach shows diffuse edematous swelling of the mucous membrane, with reddish punctate discoloration of the surface, often accentuated corrugation of the surface and considerable exuded mucus due to the hydrolysis of the alkali cyanide salts. Corrosive ulceration is rare. A similar but generally very much less marked process is found in the duodenum and occasionally even in the trachea. In the serous cavities, especially in the pericardium, punctate ecchymoses may be found and in the pia arachnoid and ventricles an edematous bloody extravasation occurs. The liver, especially if the patient has survived any length of time, may show fatty degeneration and the urine at times shows some blood.

In cases where death follows inhalation of cyanide gas, the lesion is not marked in the alimentary tract; instead there are swelling and edema of the mucous membrane of the respiratory tree with ecchymoses or hemorrhages and pulmonary edema and congestion. Lewin mentions a case of cyanide gas poisoning where degeneration of the ganglion cells, lesions of the blood vessels, thromboses and hemorrhage were found.

Histologically, in the acute poisonings, edema of the mucosa of the stomach or bronchi with circumscribed hemorrhages and leucocytic infiltration are found. Edema and miliary hemorrhages and occasionally perivascular extravasations of blood are found in the pia, brain substance and in the cord. In the chronic

cases, fatty degeneration and round cell infiltration in the small vessels, especially of the brain, are noted. Occasionally calcium deposits, hyaline degeneration and thrombus formation in the vessels are noted. In chronically poisoned animals, chromatolysis, vacuolization and degeneration of the central and peripheral nervous system have been found.

In the office of the Chief Medical Examiner of the City of New York, from January 1, 1918 to December 31, 1933, there were

TABLE 1

YEAR	HOMICIDES	SUICIDES	ACCIDENTAL (INGESTION OF CYANIDE)	ACCIDENTAL (FUMIGATION WITH CYANIDE)	TOTAL NUMBER
1918		8		2	10
1919		3		13	16
1920		10		8	18
1921		28		7	35
1922		10		4	14
1923		18		5	23
1924		18	4		22
1925		18		4	22
1926		13	1	3	17
1927		12		1	13
1928	1	18	7		26
1929	2	22	2	1	27
1930		34	2	1	37
1931		24	1		25
1932	1	44	4		49
1933		49	4	3	56
Totals.....	4	329	25	52	410

410 cases of death from cyanide poisoning. In so far as an analysis of the records permit, they are distributed as in table 1.

The deaths due to fumigation resulted from fumigation of ships, hotels and warehouses. Of the other accidental deaths, the majority were caused by mistaking the poison for medicine.

TOXICOLOGICAL ANALYSIS

Organs best suited for analysis

If the poison has been taken by mouth, the stomach contents and brain should be analyzed. Analysis of the brain is necessary for the purpose of ruling

out the possibility of the poison having been introduced into the stomach after death. If the poisoning resulted from inhalation, the lungs and brain must be examined. In cases of poisoning by inhalation, usually none, or only the very faintest trace, is found in the stomach contents. This is of tremendous importance from the medicolegal aspect.

Method of isolation of cyanide

The tissues are cooled by keeping them in an ice-box. Two hundred to 500 grams of tissue are ground up. Care should be taken to keep the tissue cold since hydrocyanic acid may volatilize if warm. If stomach contents are analyzed, usually one-fifth of the total volume of the contents is used. The ground-up tissue or the stomach contents are placed in a one liter flask and acidified with tartaric acid. The material is then distilled with steam, using a well cooled condenser the tip of which is bent to serve as an adapter and dipped into 5 cc. of 5 per cent sodium hydroxide solution in a receiving flask. The latter should be packed in ice. One hundred cubic centimeters of distillate are collected, which is ample to recover all the cyanide present, the following tests being employed.

Qualitative tests

(1) *Schönbein's test.* Suspended a strip of filter paper, impregnated with guaiac and copper sulphate, over the material in a flask, the paper being held in place by the stopper. (Dip strip of filter paper into a freshly prepared alcoholic solution of guaiac 1:10; then let dry; when dry, moisten it with dilute (1:10,000) copper sulphate solution). If color does not change, cyanide is absent and no further tests need be made. If a blue color results, cyanide *may* be present. The test is very sensitive but not specific, hydrochloric acid, nitric acid, chlorine, bromine, ozone, hydrogen peroxide, as well as some other substances also give a positive test. This test may be used as a preliminary one at the necropsy table. The following two tests must be employed since they are specific for cyanide:

(2) *Prussian blue test.* To 5 cc. of distillate, add 3 cc. of 25 per cent sodium hydroxide, then a few drops of freshly prepared ferrous sulphate solution and a few drops of ferric chloride solution. Warm a little. Let cool and add concentrated hydrochloric acid, drop-wise, until the dirty brown precipitate just dissolves; avoid excess hydrochloric acid. If cyanide is present, a deep blue precipitate (Prussian blue) appears. If only a trace of cyanide is present, a green solution results instead of a blue precipitate, but, on standing several hours, a small flocculent Prussian blue precipitate settles (sensitive to one part in 50,000).

(3) *Lieberman's test.* To 10 cc. of distillate, add 1 cc. of yellow ammonium sulphide and evaporate to dryness on the water bath. When dry, add 5 cc. of 5 per cent hydrochloric acid solution, warm a little and stir well to dissolve all of the thiocyanate that was formed during the evaporation. Let stand two hours,

then filter. To the filtrate, add 5 to 10 drops of 10 per cent ferric chloride solution. If cyanide is present, a deep red color results (sensitive to one part in 10 million).

The following tests may be used, but they are not specific for cyanide:

(4) *Vortmann's test.* To 5 cc. of distillate, add a few drops of potassium nitrite solution, then 2 to 4 drops of ferric chloride solution and then enough dilute sulphuric acid until the color of the solution becomes a bright yellow. The solution should then be boiled, after which cool and add ammonium hydroxide until all of the iron is precipitated. Filter off the precipitate. To the filtrate add a few drops of a very dilute solution of ammonium sulphide. If cyanide is present, a play of colors results, violet, blue, green, yellow.

(5) *Picric acid test.* To 5 cc. of distillate (slightly alkaline) add a few drops of picric acid solution and warm gently. If cyanide is present, a red color develops (sensitive to one part in one million).

(6) *Phenolphthalin test.* To 5 cc. of distillate, add a few drops of alkaline phenolphthalin solution (reduced phenolphthalein), then a few drops of 1:2000 copper sulphate solution. If cyanide is present, a red color develops (sensitive to one part in 20 million).

(7) *Silver test.* To 2 c.c. of distillate, add nitric acid until reaction is acid, then add a few drops of silver nitrate solution. If cyanide is present, a white precipitate of silver cyanide results.

Quantitative test

For quantitative analysis, a weighed amount of tissue is distilled, as described in the qualitative procedure. In the receiving flask, however, instead of having dilute sodium hydroxide, 20 cc. of 10 per cent silver nitrate solution are used, acidified with nitric acid. In order to make certain that all of the cyanide is isolated, distillation is continued until 200 cc. of distillate are obtained. During the distillation, the cyanide precipitates as silver cyanide. This is then filtered through a previously weighed Gooch crucible, washed, dried and weighed. From the weight of the silver cyanide, the amount of cyanide in the material analyzed is calculated as HCN.

In quantitative analysis, if the poison was taken by mouth, the entire amount of cyanide is determined in the gastro-intestinal tract. This is then multiplied by 100/98, which gives the amount in the entire body. This fraction is used because approximately 98 per cent of the cyanide, if taken by ingestion, remains within the stomach contents. If the poison was introduced by inhalation or injection, parts of all the organs and tissues are analyzed and, from these results, the amount present in the entire body is calculated.

The lethal dose of cyanide is accepted as 50 mgm., calculated as hydrocyanic acid.

The following factors interfere with the determination of the presence of cyanide:

(1) Traces of hydrogen cyanide are produced during the first few days of putrefaction, but this disappears in the later stages.

(2) Cyanides present in the tissues disappear during prolonged putrefaction and are changed to sulphocyanide.

(3) In stomach contents, where the bulk of the cyanide remains at death, putrefaction is of little importance.

(4) Embalming with formaldehyde interferes greatly in the tests, the cyanide forming condensation products with the formaldehyde.

It is, therefore, important that early toxicological analysis be made in cases of suspected cyanide poisoning. It is likewise necessary to rule out the presence of ferrocyanides, ferri-cyanides and thiocyanates before distillation is begun, because these compounds, when distilled in the presence of mineral acids, yield hydrogen cyanide.

Diffusion of the poison in a body after death has been recorded, but the process is extremely slow and it has been shown by Gettler, that, when cyanide is introduced into the stomach after death, no cyanide reaches the brain even after an interval of two months.

Although the term "cyanide of potassium" is generally employed in referring to cyanide poisoning, it is not the potassium salt but, because of its cheaper cost, the sodium salt that is used.

SUMMARY

(1) Four hundred and ten cases of cyanide poisoning are listed.

(2) The relative incidence over a period of fifteen years is noted.

(3) The pathology and mechanism of the action of cyanide is described.

(4) The tests for its chemical identification and the importance of differentiating cases of poisoning by inhalation and by ingestion are described.

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TREATMENT OF MALIGNANT NEUTROPENIA BY INJECTION OF LIVER EXTRACT*

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Since Schultz⁹ and Friedemann⁷ reported their cases of the peculiar disease characterized by ulcerating and necrosing lesions of the throat and a marked decrease in the leukocytes especially in the granulocytic cells, there have been an increasing number of cases of a similar character reported each year. This increasing incidence, together with the high mortality rate, lends interest to any suggestion that might lead to more effective treatment.

Experience in treating three cases with liver extract is the basis of this report. The usual liver preparation for oral and intramuscular treatment of pernicious anemia has been used. Intravenous injections have not been given. The usual procedure has been to give the equivalent of 300 gm. of liver by mouth and the equivalent of 100-200 gm. of liver intramuscularly each day. With the appearance of satisfactory improvement, the intramuscular injections have been discontinued and the treatment continued orally.

The first case treated was seen in March, 1930. The use of liver extract was suggested by observation of the frequent marked rise in the leukocyte count in cases of pernicious anemia receiving liver or liver extract. The average increase in the total leukocytes in fourteen cases of pernicious anemia treated with liver was 55 per cent and the average increase in granulocytes was 95 per cent. This tendency to an increase in leukocytes following liver therapy has been recorded by Murphy,⁸ Connery and Goldwater¹ and by Conner.^{2,3} The rather striking similarity between pernicious anemia and granulocytopenia has been pointed out by

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Fitz-Hugh and Krumbhaar⁵ who applied the term "pernicious leukopenia" to this condition. This similarity has been further emphasized by Beck¹ who stated that it seemed reasonable to suppose that the primary lesion is not in the bone marrow but in the organ or tissue which gives rise to the substances that keep maturation of the granulocytes regulated to a normal level. It is possible that the liver is this organ, and that in granulocytopenia there is a deficiency condition as in pernicious anemia. Foran, Sheaff and Trimmer⁶ report five cases treated with liver extract given orally, intramuscularly and intravenously. All of these cases recovered. One died in a second attack complicated by pneumonia.

CASE REPORTS

Case 1. In March, 1930, a white woman, age 38, was seen in her home. She had been ill five days. The onset had been with chill, high fever, prostration, extreme nervousness and soreness of the mouth. A blood count made by her attending physician two days before showed 600 leukocytes; the differential formula was not available.

Her past history indicated that her general health had been fairly good until one year before. She was married and had had one child twelve years before that died five days after birth following a difficult labor. She developed bladder irritation in September, 1928, and suffered from this off and on during the fall and winter, with frequency, burning, and blood in the urine. During the winter she was operated on for fissure in ano. In February, 1929, she had an acute attack with chill, high fever, and severe soreness of her mouth. According to her statement her lungs were congested. She was acutely ill for three weeks and continued to feel badly all summer. Her health had been good during the winter months of 1930.

When seen in March, 1930, she appeared extremely ill. She was prostrated and complained of extreme weakness and nervousness. Her temperature had been ranging from 101 to 103°. The skin was pale. There was no icterus of the skin or sclerae. The tongue and the mucous membranes of the mouth were very red. The gums were swollen, tended to bleed, and were covered with a thin, white membrane. There were no areas of necrosis. The neck was somewhat swollen on both sides and the cervical glands were moderately enlarged. There was no ulceration about the rectum or vagina. The spleen was palpable below the costal margin. The general physical examination was otherwise negative. Laboratory findings were as follows: hemoglobin 52 per cent; erythrocytes, 2,270,000; leukocytes, 2,300; polymorphonuclear neutrophils, 48.0 per cent, small monocytes, 42.0 per cent, large monocytes, 9.5 per cent;

transitionals, 0.5 per cent; platelets, 267,000; reticulocytes, 0.4 per cent; van den Bergh reaction, delayed direct, quantitative 1.8 mg. per liter; blood culture, negative. Smears from the mouth and throat showed no Vincent's organisms. Wassermann test was negative.

The picture at that time did not suggest pernicious anemia but she was given liver extract by mouth, receiving the equivalent of 100 gm. of liver three times a day. There was a rapid improvement in the patient's condition. The mouth cleared promptly, the soreness and swelling of the neck subsided, and the temperature returned to normal in five days. Not much significance was attached to the possible effect of liver therapy at the time, as it was thought that the patient was undergoing a spontaneous recovery. Between March 27, 1930, and July 28, 1930, her erythrocytes gradually increased from 2,270,000 to nearly

TABLE 1
CASE 1

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	POLY-MORPHO-NUCLEARS	EOSINOPHILES	BAKOPHILES	SMALL MONO-NUCLEARS	LARGE MONO-NUCLEARS	TRANSITION-ALS
1931	per cent			per cent	per cent	per cent	per cent	per cent	per cent
April 23*	92	4,090,000	7,000	68.5	3.0	0.5	22.5	4.0	1.5
July 15†	90	4,410,000	1,200	12.0		2.0	62.0	20.0	4.0
July 16			1,060	2.0		0.5	80.0	12.5	5.0
July 18	74	3,640,000	3,500	38.0		0.0	52.5	7.5	2.0
July 20	73	3,500,000	3,700	31.0		0.0	57.5	9.0	2.5
July 26	84	4,420,000	7,600	67.0		1.5	25.5	6.0	

* One vial liver extract every day.

† Three vials, intramuscularly, every day.

5,000,000, her hemoglobin from 52 per cent to about 90 per cent. There were in general no significant blood findings except a myelocyte count of 14 per cent on March 29 followed a few days later by a reticulocyte increase, as high as 10.9 per cent.

The patient continued well during the remainder of 1930, all of 1931, and up to July, 1932, except for one episode in May, 1930, when, following temporary discontinuance of the liver, her leukocytes fell to 3,200, the granulocytes to 44.0 per cent, and the lymphocytes increased to 51.0 per cent. On return of treatment by liver by mouth, the balance was restored. During this time she maintained a normal blood picture on 1 to 2 vials of liver extract each day (hemoglobin, 90 per cent; erythrocytes, 4,500,000 to 5,000,000; differential count, normal.)

On July 15, 1932, the patient stated that for some time she had been having

attacks of generalized aching at intervals of two weeks, accompanied by slight fever, and followed by severe sweats. Two days before she had had such an attack. On examination, her temperature was 99.3°; there was no reddening or ulceration of the gums or throat, and no soreness or enlargement of the glands of the neck. The general physical examination was negative. The blood count, however, showed hemoglobin 90 per cent; erythrocytes, 4,410,000; leukocytes, 1,200; polymorphonuclears, 12.0 per cent; basophiles, 2.0 per cent; small monocytes, 62.0 per cent; large monocytes, 20.0 per cent; transitionals, 4.0 per cent. The following morning her temperature was 101°. The gums were slightly swollen and sore. The glands in the neck were distinctly enlarged and there was general soreness of the neck. The leukocytes numbered 1,060; polymorphonuclears, 2.0 per cent; basophiles, 0.5 per cent; small monocytes, 80.0 per cent; large monocytes, 12.5 per cent; transitionals, 5.0 per cent. The liver extract was increased to three vials by mouth each day, and on the 16th, 18th, and 20th she received 3 cc. of liver extract intramuscularly. The course of her blood response is shown in table 1. It is to be noted that the fall in the leukocytes preceded the drop in erythrocytes and hemoglobin by several days, and that in the restoration of the normal blood picture the same relationship prevailed. This illustrates the importance of the time element in the appearance of anemia in these cases.

From then on she remained in very good health on three vials of liver by mouth each day. In January, 1934, the liver dosage by mouth was reduced to two vials a day. On March 16, 1934, she presented herself complaining of weakness, nervousness, exhaustion, rapid heart action, and soreness of her gums. The gums were red and spongy. There was no atrophy of the papillae of the tongue, and no enlarged glands. The temperature was normal, and the pulse 120. The leukocytes had fallen to 3,000; hemoglobin 76 per cent; erythrocytes, 3,610,000. She was given 3 cc. of liver extract intramuscularly on three successive days and put back on three vials of liver extract by mouth each day. On March 22nd the hemoglobin was 84 per cent; erythrocytes, 4,060,000; leukocytes, 4,300; reticulocytes, 4.2 per cent.

This patient has been observed over a period of four years. The onset of her illness was acute and stormy, with chill, high fever, ulcerative stomatitis, and enlargement of glands of the neck. During this attack her leukocytes fell as low as 600. In one subsequent attack the leukocytes went down to 1,060. In this attack the drop in the leukocytes was detected before the onset of the local symptoms. This attack apparently responded to intramuscular liver extract. In between the acute attacks she had pursued the course of a case of true pernicious anemia. It is worthy of attention that during her initial attack she developed during her period of recovery a myelocytic crisis with 14.0 per cent myelocytes in the blood, and that there was also an increase of her reticulocytes to 10.9 per cent.

Case 2. A white man, age 63, was first seen on February 24, 1932. At that time he had been ill for eight weeks with soreness and stiffness in his joints and the muscles of the legs. He had a normal blood picture: hemoglobin 84 per cent; erythrocytes, 4,170,000; leukocytes, 8,100; polymorphonuclears, 65.5 per cent; eosinophiles, 0.5 per cent, small monocytes, 30.5 per cent; large monocytes, 1.0 per cent; transitionals, 2.5 per cent. His Wassermann and Kahn reactions were positive and he was referred to his family physician for treatment. During March he received three injections of neoarsphenamine; 0.3 gm., 0.45 gm., and 0.6 gm. There was no reaction following any of these injections.

On April 9, 1932, he was seen at home. He had been feeling badly for two days and was complaining of weakness, malaise, fever, and sore throat. On examination the only noteworthy findings were in the throat and neck. The throat was swollen and congested and covered with a dirty, grayish membrane resembling a diphtheritic membrane. Smears taken from the throat were negative for diphtheria bacilli on direct examination and by culture. There was moderate swelling and tenderness on both sides of the neck. The blood count showed 2,900 leukocytes with 2 per cent granulocytes, 76 per cent small mononuclears, 19 per cent large mononuclears, and 3 per cent transitionals. Liver therapy was started at once. At first he was unable to take the extract by mouth on account of swelling and soreness of the throat. He was given, however, 3 cc. of liver extract intramuscularly each day for three days, and then every other day for three doses. There was an immediate subsidence of the swelling and soreness of the throat, return of temperature to normal, and rapid recovery from malaise and weakness. Along with this went an improvement of the blood picture. Over a period of twelve days his leukocytes increased from 2,900 to 6,200, and the granulocytes from 2 per cent to 45 per cent.

During May and June there was some variation in the administration of liver, and both the total leukocytes and the percentage of granulocytes fell again; on June 15, 1932 there were 3000 leukocytes, of which 32 per cent were polymorphonuclear neutrophils. Following the resumption of liver therapy the blood count reached an approximately normal level in August, 1932. This patient has remained well since that time.

In this case the condition probably arose as a result of arsenic therapy. Clinically and hematologically the primary and secondary cases are indistinguishable and, except for the elimination of the primary factor, the therapeutic indications are the same. This patient pursued a rather chronic course, but it may be of significance that more rapid and complete improvement took place when the liver therapy was better regulated.

Case 3. A white woman, age 39, was admitted to the hospital on July 8, 1933. She had been ill for four days with general malaise, fever, chilly sensa-

tion, exhaustion, and sore mouth and throat. Her temperature had been ranging as high as 103°. She gave a history of what she considered two similar attacks, one four years before, and one two years before. Both of these attacks had been diagnosed as trench mouth. There was nothing else significant in her previous history except that she had lost her husband eight months before and had been very nervous and depressed. As a result of this, during the past eight months she had used sedatives of the barbituric acid series to a considerable extent on account of insomnia.

Examination revealed a well nourished woman, depressed and very nervous. Her color was good. There was extensive ulceration beneath the tongue. The gums were swollen, bluish red in color, and covered with a thin, white membrane that stripped off easily. In two places on the hard palate there were discrete ulcerated areas. The throat was red. The neck was swollen on both sides,

TABLE 2

CASE 3

DATE	HEMO- GLOBIN	ERYTHRO- CYTES	LEUKO- CYTES	POLY- MORPHO- NU- CLEARS	BASO- PHILES	SMALL MONO- CYTES	LARGE MONO- CYTES	TRANSI- TIONALS
1933	<i>per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
July 8	84	4,210,000	1,200	18		58	18	6
July 9	90	4,620,000	2,200	28		69	1	2
July 10	89	4,860,000	2,500	24		70	4	2
July 11	88	4,610,000	3,700	53	1	38	6	2
July 12	87	4,460,000	4,000	55		33	7	5
July 13	84	4,410,000	4,500	60		38		2
July 14	85	4,470,000	6,700	54		38	5	3
July 15	84	4,410,000	5,700	70	1	25	3	1

especially the left, which was rather brawny, and the glands at the angle of the jaw were enlarged on both sides. The neck was very tender to palpation. The head was normal, the lungs clear, and the spleen not enlarged. The vaginal tract was negative.

On admission her temperature was 101°, on the second day 102°, and on the third day was normal and remained so thereafter except for a slight afternoon rise for two days. The blood examination on admission showed a leukocyte count of 1,200 with 18 per cent granulocytes. The subsequent course of the blood counts is shown in table 2. Blood culture was negative. Smears from the mouth showed no fusiform bacilli. She was given 3 cc. of liver extract intramuscularly and put on liver extract by mouth three times each day. The second day she received two injections of liver extract, the third day one, and thereafter was continued on the extract by mouth. By the seventh day the blood picture had returned to normal and remained so thereafter. With the

fall in temperature and improvement in the blood picture, there was a rapid subsidence of symptoms. The mouth cleared; the swelling and tenderness in the neck disappeared and there was an immediate return of a sense of well-being.

This patient made a very rapid recovery while taking liver by mouth and intramuscularly. While the change in the blood picture was rapid and striking, the clinical improvement was even more impressive. The history would indicate that she had experienced two other attacks from which she recovered spontaneously, neither one of these, however, was as severe as the last attack. It is possible that she again recovered spontaneously, but the rapid change in her clinical condition following liver therapy strongly suggested a causal relation. There has been no opportunity to observe this patient since discharge from the hospital eight days after admission, but a recent communication from her doctor indicates that she has remained well.

The three cases reported have recovered and are apparently well at the present time. One of them has to continue on a regular ration of liver. If this is diminished or discontinued she tends to revert, not only to a pernicious type of blood picture, but to a leukopenic state as well.

It is, of course, realized that in reporting results in these three cases no very valid conclusions can be drawn. It is possible that all of these cases would have made spontaneous recoveries without the aid of liver extract. It is felt, however, that the results obtained are suggestive and would seem to justify the further use of liver extract in the treatment of granulocytopenia.

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EDITORIAL

THE PASSIVE TRANSFER METHOD OF ALLERGIC DIAGNOSIS

During the last ten years there has been a very definite tendency toward the specialization of allergic practice. A variety of factors have made this necessary. Allergy is a rapidly growing new branch of medicine in which the advances have been made chiefly by those investigators who have had the opportunity to devote practically their entire time to the subject. At first it appeared as if allergic diagnosis would be a very simple laboratory procedure in which certain tests were applied directly to the patient's skin and the answer was thereupon correctly revealed to anyone who chose to read the reactions.

So many variables, however, play a part in the elicitation of the skin reaction that this is no longer considered, in any sense, infallible. Many supplementary methods of investigation have since been developed and it is quite true that for the adequate study of the allergic diseases, one's full time is often required.

At the same time allergy has been found to dominate or to color such a wide variety of clinical manifestations and such a relatively large proportion of the population that the time will come when the man not primarily interested in allergic investigations must give certain of his patients the benefits of allergic study. While, today, there is scarcely room for argument that the investigator who performs the skin tests should be the person who is making the entire allergic study in the broader sense, at the same time convenience or necessity often dictates that the general man have this portion of the allergic work done by someone appropriately equipped therefor. As a consequence in certain sections, the clinical pathologist is called upon to do the routine sensitization tests, reporting the results of study to the attending physician.

Although the direct method of testing usually gives a large

amount of positive information and remains at present the basic point of departure for allergic studies, its shortcomings may be briefly summarized under three categories: the appearance of a certain proportion of false positive reactions, reactions to allergens which are subsequently shown to have no clinical significance in the particular case under investigation; false negative reactions, in which for one reason or another information that should have been of value was not elicited; and inappropriate reactive surface, in which the patient's skin for one reason or another cannot be used. This occurs especially in infants, in individuals with extensive dermatitis, in cases of dermatographia, in individuals too ill to be subjected to the inconvenience of skin testing, and in those who find it necessary to take adrenalin so continuously that the skin reaction is not reliable.

One of the several methods available for counteracting any or all of these disadvantages is the passive transfer method, which, incidentally, is truly a laboratory procedure. This method has been developed on the basis of the familiar Prausnitz-Kustner reaction, in which antibodies or reagins may be transferred from the serum of an allergic individual into the skin of a non-allergic recipient, thereby rendering this skin area sensitive for a short time to the particular allergen in question. The method has been popularized in this country by Matthew Walzer who describes it in detail in "Asthma and Hay Fever in Theory and Practice"* and in a number of subsequent contributions.

Briefly the method consists in obtaining 5 or 10 cc. of the patient's blood under aseptic precautions, separating serum or preferably plasma; ultra-filtration; and its introduction intracutaneously into selected skin areas of a non-allergic recipient. Walzer uses no preservative although the writer has found no disadvantage to the addition of 0.5 per cent phenol. The sensitizing dose should be rather large (0.07 cc. to 0.1 cc.). An area of perhaps two inches diameter becomes sensitized and remains so for upwards of four weeks. Walzer recommends the arm, the

* Coca, A. F., Walzer, Matthew, and Thommen, A. A.: *Asthma and hay fever in theory and practice*. Springfield: Thomas, 1931, pp. 851.

writer prefers the back. Usually a mole or other landmark is available as a point of orientation, from which one side of the back is checker-boarded with the intracutaneous injections, two inches apart vertically and horizontally. Twenty-five or more areas may be sensitized.

The trauma of the procedure is apt to produce a refractory period which may last twenty-four or forty-eight hours, or longer. By preference the actual testing is therefore not done until the third or fourth day after preparation.

At this time the test solutions are introduced, in the usual 0.01 cc. or 0.02 cc. dosage, at points about one quarter inch from the sites of the original needling. Control tests are applied in the same dosage at symmetrical points on the non-sensitized half of the back.

Readings are made usually at the end of twenty or thirty minutes. Each test substance is read by comparison with its control reaction. Although, occasionally, large urticarial reactions result, any real degree of difference from the control is of significance. Not infrequently some of the control reactions are positive, indicating either an unsuspected sensitiveness on the part of the recipient, or an irritating test solution. Such reactions cannot be interpreted and should, if feasible, be repeated on another recipient. When a control reaction is positive and the passively sensitized area is very much more strongly positive, this is usually indicative of a positive reaction.

After an additional three or four days the same areas may again be used for further testing. Areas that have given positive reactions on the first testing should however not be used again, and most particularly, should not be used with the same test solution that gave the original positive reaction, since the antibodies have been exhausted for this particular antigen.

The advantages claimed by Walzer for this method are as follows: so-called false positive reactions are avoided since the reacting body must be present in the patient's blood for the reaction to be positive by this method; the method may be used in cases in which the condition of the patient's skin precludes satisfactory direct testing, such as in diffuse eczema with or without

secondary infection, ichthyotic skin, and those persons scarred by constant scratching in inflammation; urticaria, dermographism; contagious skin infections such as impetigo; and in the other conditions mentioned above.

—WARREN T. VAUGHAN.

NEWS AND NOTICES

Announcement has been received of the organization of the American Association for the Study of Neoplastic Diseases. It is planned that the Society will hold four meetings a year and that the September meeting will be held at the Mayflower Hotel in Washington, September 6 to 8. The program consists of two sessions with lantern slide demonstrations of microscopic pathology, two sessions on radiologic diagnosis, and three sessions on surgery and radiation therapy in neoplastic diseases. All persons interested in the work of the Association are invited to attend the meeting.

Dr. M. Pinson Neal served as Visiting Professor of Pathology and Bacteriology at the University of Tennessee College of Medicine and as Acting Pathologist to the Memphis General Hospital during the summer quarter at Memphis, Tennessee.

The International Society of Radio-Biology will sponsor an International Congress of Electro-Radio-Biology to be held in the Doges Palace in Venice, September 10 to 15. The Congress will be presided over by Marquis Guglielmo Marconi. The object of this Congress is to invite for a discussion, physicists, chemists, biologists, naturalists, and physicians, on biological actions of all radiations, in order to coördinate the respective investigations. A general invitation has been made to all others interested in the subject.

THE SEROLOGIC CONFERENCE

The Society will be interested to learn that Surgeon-General Cumming of the United States Public Health Service has included the "serologic conference" in the budget of that government service for the coming year. The committee that reported on this matter at the Cleveland meeting was continued with power

to act. Accordingly, Dr. Vonderlehr of the U. S. P. H. Service was made chairman with Drs. Simpson and Sanford on the committee to represent the A. S. C. P. Two syphilologists are to be chosen by these three members and this committee of five will have charge of the plan to evaluate independent serologic procedure for the diagnosis of syphilis in the United States.

The following notice is to be published in various medical journals:

The United States Public Health Service is coöperating with the American Society of Clinical Pathologists in the drafting of a plan to evaluate independently serologic procedure for the diagnosis of syphilis in this country. Briefly, the plan contemplates the collection of specimens of blood from at least 1,000 individuals and the distribution of comparable specimens to the laboratories of serologists who have described an original modification of a complement-fixation or precipitation test for the diagnosis of syphilis. The donors of the specimens will be carefully selected so as to measure both the specificity and sensitivity of the serologic procedure. The sending of specimens to workers at considerable distance from the point of collection will be expedited by the use of the most modern transportation facilities, while the delivery of specimens to nearby serologists will be delayed so as to make the delivery time approximate that for those workers at the more remote points.

A committee of five members consisting of two specialists in the field of clinical syphilology, two members of the American Society of Clinical Pathologists, and one officer of the United States Public Health Service will organize the plan of study and, after all laboratory reports have been submitted by participating serologists, will interpret the results on the basis of clinical findings. The collection of the specimens will begin about December 1, 1934, and a number of serologists will be invited to take part in the evaluation scheme.

It is possible that the name of some serologist who has described an original modification of a test for syphilis may have been inadvertently omitted. Any serologist desiring to participate will be extended an invitation upon presentation of suitable proof

as to the originality of his modification of a serologic test. A brief description of the plan will also be sent to those workers who may be interested.

Correspondence should be addressed to the Surgeon-General, United States Public Health Service, Washington, D. C.

THE ETIOLOGY OF GRANULOPENIA (AGRANULOCYTOSIS)

WITH PARTICULAR REFERENCE TO DRUGS CONTAINING THE
BENZENE RING*†

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In previous communications ^{11, 12, 13, 14} we have presented evidence to indicate that the chief etiologic factor in granulopenia is the administration of certain classes of drugs, particularly those which contain the benzene ring as their central nucleus. In June, 1931, before the American Society of Clinical Pathologists, we presented evidence tending to show that the injection of pure benzene is followed by clinical granulopenia in rabbits and also that the action of benzene on the hematopoietic tissue is more likely due to one of its oxidation products rather than to the drug itself. At that time we called attention to the fact that eight of the nine patients with granulopenia had been given large amounts of coal-tar benzene ring drugs prior to the clinical onset.

In 1931 one of us¹¹ published a report of a patient who had been given so much phenacetine over a three year period that she was admitted to the hospital with a severe methemoglobinemia and an acute fulminant attack of granulopenia. We¹⁴ reported eleven cases of acute fulminant granulopenia in all of whom the clinical onset had been preceded by the administration of benzene ring drugs and presented the hypothesis that this disease is due mainly to the administration of that type of drug, attempting to formu-

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934. The Ward Burdick Award was made to Dr. Kracke for his work on granulopenia at the annual convention.

† This work aided by a grant from the Therapeutic Research Committee of the American Medical Association.

late a series of oxidation reactions in which it could be shown that these drugs can be oxidized to a common end product. We have called attention also to the high incidence of this disease in nurses and physicians and relatives of physicians and believe that this is brought about by the more common usage of these drugs in people of the medical group.

It is the purpose of this paper to present further evidence that our original conception of granulopenia was correct, and we propose to show; first, that granulopenia, based on a review of 1385 death reports, is a disease that affects nurses, physicians and medical people more than any group in this country; secondly, that certain drugs of the benzene ring class are capable of being easily oxidized to toxic products which are capable of producing granulopenia in rabbits.

Since our original communication on this subject the question of drug relationship to granulopenia has received confirmation from the important work of Madison and Squier¹⁶ who reported fourteen cases of granulopenia; six of the patients had taken allonal, four amidopyrine, and the other four various drugs in combination with amidopyrine. They also showed that in six of these patients amidopyrine was used therapeutically after the onset of the disease and all of these died, while in the remaining eight, amidopyrine was prohibited and only two died. Furthermore, they were able to induce typical attacks of granulopenia by the administration of amidopyrine; on administering the drug to nineteen rabbits under variable conditions one animal developed granulopenia on the thirtieth day. This report apparently has stimulated many others to similar observations, for in the past few months Hoffman et al.⁸ have reinvestigated their fourteen cases of granulopenia and found that thirteen of these patients had taken amidopyrine and that the onset in one was preceded by the administration of dinitrophenol. Randall¹⁹ has reported the sudden development of granulopenia in a physician following the use of a barbiturate and amidopyrine. Zininger²³ reported granulopenia in two sisters although she did not mention any association with drugs. At our request she later investigated the drug history and found that both of these patients had taken

large and undetermined quantities of amytal compound preceding the clinical onset.²⁴ Costen⁴ reported three cases in which he stated his patients were women of "sedentary lives and nervous temperament," who required daily use of sedatives to obtain sleep and pointed out that one of his patients took large doses of aspirin, phenacetine and bromides; that another used allonal daily for several months, while the third was known to have taken large quantities of various sedatives.

The relation of drug therapy to granulopenia is being studied in Copenhagen, Denmark since Holten et al.⁹ have reported five cases developing the disease while in the hospital being treated for other disorders, and in every instance had been given amidopyrine in the course of their treatment. They referred to a similar case by Videbech.²¹ Andersen² reported a patient that had taken a mixture of amidopyrine and a barbiturate daily for five months and stated that his patient had recurrences following the administration of barbipyrine (compound containing amidopyrine), with recovery after the drug had been discontinued. Thus, at this time, there have been reported fifty cases of acute fulminant granulopenia in which various benzene ring drugs are incriminated as etiological agents with the chief interest centering on amidopyrine (see table 1).

Of these fifty patients forty-six have taken amidopyrine either alone or in combination with other drugs. Most of them have received the drug in combination with some form of barbiturate, while others have not received barbiturates. The onset of one was preceded by the administration of dinitrophenol⁸ and in our series of eleven cases two had received large quantities of phenacetine; we reported one patient who took large amounts of acetanilid. However, this patient has since been reinvestigated and was found to have received amidopyrine also. We believe, therefore, that acetanilid can be ruled out as an etiological drug since there is no report of any case of granulopenia following the use of that drug alone. Since the publication of our last report we have seen a dentist with the acute fulminant type of the disease accompanied by complete loss of granulocytes, who had taken large quantities of phenacetine and we could not elicit any history of

TABLE 1

CASES OF GRANULOPENIA REPORTED WITH SUSPECTED DRUG ETIOLOGY

AUTHOR	CASE NUMBER	DRUG
Madison and Squier.	1	Allonal
	2	Allonal
	3	Allonal
	4	Allonal
	5	Allonal
	6	Sodium amytal-amidopyrine
	7	Amidopyrine
	8	Neonal-amidopyrine
	9	Amidopyrine
	10	Amidopyrine
	11	Amidophen-phenobarbital
	12	Amidopyrine
	13	Allonal
	14	Amytal-amidopyrine
Hoffman, Butt and Hickey.....	1	Amidopyrine
	2	Amidopyrine
	3	Amidopyrine
	4	Amidopyrine
	5	Amidopyrine
	6	Amidopyrine
	7	Amidopyrine
	8	Amidopyrine
	9	Amidopyrine
	10	Amidopyrine
	11	Amidopyrine
	12	Amidopyrine
	13	Amidopyrine
	14	Dinitrophenol
Krooke and Parker	1	Peralga
	2	Amidopyrine
	3	Amidopyrine-phenacetine
	4	Acetanilid
	5	Amidopyrine
	6	Various benzene drugs
	7	Neocarphenamine-phenacetine
	8	Phenacetine
	9	Phenacetine
	10	Allonal-amidopyrine
	11	Amidopyrine
Randall	1	Amidopyrine-barbiturates
	1	Amidopyrine
Helton, Nielsen and Transbol	2	Amidopyrine
	3	Amidopyrine
	4	Amidopyrine
	5	Amidopyrine

Some with and
others without
barbiturates

TABLE 1—*Concluded*

AUTHOR	CASE NUMBER	DRUG
Costen.....	1	Aspirin-phenacetine
	2	Allonal
	3	Large quantity sedatives
Zinninger.....	1	Amytal compound
	2	Amytal compound
Pouzin-Malegue.....	1	Arsphenamine
Cassaute, et al.....	1	Arsenobenzol
Lande.....	1	Solganol
Jacquelin and Allanic.....	1	Crisalbine
Achard, et al.....	1	Solganol-crisalbine
	2	Solganol-crisalbine

administration of amidopyrine. It appears to us at this time that amidopyrine and phenacetine are incriminated in the production of this disease. Dinitrophenol might be added to these since this is a drug of the same class in so far as its benzene ring structure and oxidation potentialities are concerned.

There are a few references in the literature concerning the effect of drugs on the leukocyte count. Turley and Shoemaker²⁰ reported the effect of quinine and luminal on the leukocyte count, but since they gave only one grain of quinine daily to adults, the results would seem to be of little value. They also gave dogs two and a half grains of luminal daily for five weeks with a slight decrease of the leukocyte count during the first week. Nye and Barrs¹⁷ found no effect on rabbits following the administration of amytal. More recently Watkins²² has presented a brief summary of thirty-two cases of granulopenia from the Mayo Clinic and found that thirteen of these had received amidopyrine while eleven had received drugs of the barbiturate class, and in eight no history was obtained. In this connection, we would like to emphasize that obtaining an accurate history relative to the previous use of drugs in a patient with granulopenia is an extremely difficult task. In our experience, it is almost impossible to secure a history of any value from the relatives and friends of a patient who has long since been dead.

Hardwick and Randall⁷ have studied the effect of pentobarbital

sodium on the leukocyte count of fifty-nine pregnant women and reported no depressing effect from this drug. It must be noted that these authors used a straight chain barbiturate and not a drug containing the benzene ring. It can be seen, therefore, that the available evidence points toward the incrimination of the benzene ring and the exclusion of the barbiturates. It is impossible to state at this time whether or not the action of the benzene ring drug is more effective by its combination with a barbiturate.

There are many reports in the literature of cases of granulopenia following the use of arsphenamine and neoarsphenamine, as the report of Cassaute et al.³ in which a typical case developed following two injections of arsenobenzol, and that of Pouzin-Malegue¹³ in which the disease developed after the administration of arsphenamine. It has long been known that arsenical preparations are capable of producing profound hematopoietic depressions. Most of these, however, are recorded in the literature under the diagnosis of aplastic anemia. There is ample evidence that in an occasional instance the administration of this type of drug produces a depression limited to granulocytes.

Concerning the combination of amidopyrine with the barbiturates, as seen in allonal and peralga, the Council on Pharmacy and Chemistry states that these are not chemical compounds but merely physical unions and mixtures of two drugs and that in all probability as soon as they reach the stomach they are broken down into their original components.

Relative to peralga, the Council on Pharmacy and Chemistry makes this significant statement,⁵

If barbital or amidopyrine is placed in an oven at 100°C. no apparent change takes place; but when mixed fusion occurs such as happens in a depressed melting point determination, there is formation of yellow color and amine odor. In this manner there is formed a relatively small amount of a decomposition product, or probably products, not identified.

The above statement appears to us an important clue in the production of granulopenia and we submit evidence in this paper that this yellow product with an amine odor is an oxidation and

decomposition product and in all probability is quinone and that these products, when injected into rabbits, are capable of producing granulopenia. It would appear that there is no justification for combining amidopyrine, an analgesic, with barbitol, a hypnotic, when there is evidence that dangerous toxic products are evolved in the process.

There have been reported cases of granulopenia that have arisen presumably following the administration of a class of drugs, commonly referred to as "gold salts," used in the treatment of tuberculosis. Reports of granulopenia following these drugs are quite common in France where they have the widest usage; Lande,¹⁵ Jacquelin and Allanic,¹² Emile-Weil and Bousser,⁶ Achard et al.¹ The report of the latter is of particular interest. These authors treated a patient with repeated injections of a gold salt known as crisalbine with no depression of the leukocyte count, which at this time was 11,400. This was followed by four injections of solganol with the leukocyte count dropping to 1200 with almost complete granulopenia. Another patient received ten weekly injections of crisalbine with no effect on the leukocyte count, but after receiving ten injections of solganol the count dropped to 2200 with complete granulopenia and death of the patient. This report is important since crisalbine does not contain the benzene ring, whereas solganol does contain the ring with the attached amine group, while both drugs contain the gold salt in its chemical structure.

As we have pointed out before,¹⁴ the gold salts, arspenamine, amidopyrine and phenacetine have certain structures in common, namely, the benzene ring with the attached NH_2 , or amine group. This group merely facilitates the ease of oxidation. In the case of arspenamine the benzene ring with the attached amine group is probably responsible for the leukocyte depression. It is of interest that most of these drugs were introduced about 1922 and have had their widest usage since that time, and granulopenia was first observed in the same year. Under the designation of amidopyrine, this drug has been on the market for twelve years. Phenacetine was introduced about twenty-four years ago. Empirin compound, a mixture of acetylsalicylic acid, phenacetine and

caffeine, was introduced in 1923. Allonal was marketed in America in 1922 and peralga in the same year. The use of gold salts is comparatively recent. There are many other drugs that are in current use by the medical profession and by the laity that have this same essential structure, but are not mentioned in this paper since we confine our discussion only to those that have been reported as being causative in the production of granulopenia.

THE INCIDENCE OF GRANULOPENIA IN THE UNITED STATES WITH PARTICULAR REFERENCE TO OCCUPATIONAL STUDIES

In previous communications we have pointed out the apparent high incidence of this disease in physicians, nurses and medical people based on case reports in the literature. In order to verify this, we procured, from the United States Bureau of Vital Statistics, copies of the death reports of all cases under the heading of "agranulocytosis" and "agranulocytic angina" that were available. We were supplied with 1385 death certificates of this disease for the years 1931-32-33. Due to incomplete data seventy-one of these were excluded and our statistical studies are based on 1314 cases. A survey of the American literature reveals only 600 cases reported from 1922 to 1933. It is impossible to determine how many cases have occurred since this disease was first reported, but no doubt the number must run into the thousands. Furthermore, it is reasonable to assume that many patients have died with the diagnosis unrecognized.

Granulopenia had its first recognition origin at about the same time in Germany and the United States and probably occurs in about the same proportion in these two countries. We have pointed out before its rarity in England and this still remains true. It is of interest that many of the drugs have not had the wide spread and promiscuous use in that country as in the United States.

The disease, still remains one mainly of the white race; colored people rarely use the type of drugs that are incriminated in this list.

Table 2 shows the occupation distribution in the various groups. It is very evident from a study of this table that granu-

lophenia is far more prevalent among physicians, nurses, dentists, druggists, laboratory technicians, hospital orderlies, etcetera, than in any other group of people in the country. Although by far the largest number of cases are housewives, this group constitutes the largest population group but the rate of its occurrence in that group is 1.2 per hundred thousand as compared with 7.7 per hundred thousand of the medical population. We feel quite sure that the same figures will hold for relatives of physicians. The occurrence of the disease in members of doctors' families has been brought to our attention too often to be ignored.

TABLE 2
OCCUPATION

Occupational distribution per 100,000 population during 1931-1932-1933

Physicians.....	12.4
Nurses.....	8.5
Dentists.....	5.6
Druggists.....	2.9
Laboratory technicians.....	12.0
Hospital orderlies.....	5.0
Total medical group.....	7.7
Farmers.....	0.2
Lawyers.....	1.8
Ministers.....	2.0
School teachers.....	2.2
Stenographers.....	0.7
U. S. population as a whole.....	1.07
Housewives.....	1.2

In table 3 it can be seen that of the 236 death certificates of males in which the occupation was stated, physicians comprised the largest group and the same is true of nurses when housewives are excluded. For example, the condition is far more prevalent among nurses than among female school teachers and stenographers, and more prevalent among physicians than among lawyers and ministers. There can be little doubt but that the disease is prevalent among medical people and what ever theory of etiology receives final acceptance, in our opinion it must also explain this peculiar relation to the medical profession. We

have seen few physicians who do not have a package of allonal, amytal compound, peralga, or other such drugs lying on their desk within easy reach. It is easy for the doctor and certainly common practice when members of his family become ill to reach for one of these "newer and better drugs," with which he has been circularized and detailed. The same is true of the nurse in the course of her hospital duties.

TABLE 3
OCCUPATION AND SEX
Cases in which the occupation is stated (1314 cases)

Males.....	236
Physicians.....	19
Dentists.....	4
Druggists.....	2
Hospital employees.....	5
Laboratory technician.....	1
Total medical group.....	37
Total medical group.....	16%
Females.....	159
Nurses.....	24
Pharmacists.....	1
Hospital employees.....	6
Physician.....	1
Total medical group.....	32
Total medical group.....	20%

OXIDATION OF DRUGS

It is our belief that the depressant action of this class of drugs on granulopoietic activity is due to one of its oxidation products and it is theoretically possible for all of these drugs to be oxidized to quinone. In a previous paper¹⁴ we have presented this conception in detail and outlined an hypothesis illustrating the possible reactions by which the drugs of the benzamine group may be oxidized to the common end-product, quinone.

A series of experiments were designed to reproduce as nearly as possible the conditions of pH and temperature to which these drugs would be subjected in their passage through the upper

gastro-intestinal tract with the addition of a mild oxidative reaction. Certain barbiturates which were also oxidized under identical conditions served as controls.

Amidopyrine, phenacetine, acetanilid, acetylsalicylic acid, amytal and barbital, were each dissolved or suspended in 0.5 per cent HCl, in 0.5 per cent NaOH, and in neutral distilled water, incubated in a water bath at 40 to 45°C., and subjected to free flowing oxygen for periods of time varying from ten to thirty-

TABLE 4

DRUG SOLUTION	4 HOURS	12 HOURS	36 HOURS
Amidopyrine—0.5% HCl.....	Slate to Yellow	Deep Yellow	Amber
Amidopyrine—0.5% NaOH.....	None	None	None
Amidopyrine-neutral.....	None	None	None
Phenacetine—0.5% HCl.....	None	None	Yellow
Phenacetine—0.5% NaOH.....	None	Yellow	Intensified
Phenacetine-neutral.....	None	None	None
Acetanilid—0.5% HCl.....	None	None	None
Acetanilid—0.5% NaOH.....	None	None	None
Acetanilid-neutral.....	None	None	None
Acetylsalicylic—0.5% HCl.....	None	Violet	Violet
		(Needle crystals)	
Acetylsalicylic—0.5% NaOH...	None	None	None
Acetylsalicylic-neutral.....	None	None	None
Amytal—0.5% HCl.....	None	None	None
Amytal—0.5% NaOH.....	None	None	None
Amytal-neutral.....	None	None	None
Barbital—0.5% HCl.....	None	None	None
Barbital—0.5% NaOH.....	None	None	None
Barbital-neutral.....	None	None	None

six hours. Table 4 shows the color changes taking place with the approximate reaction time.

In those positive reactions indicated above, the yellow color was identical with that seen in a weak aqueous solution of quinone and there was a definite quinone odor in the flasks. The colored compound was soluble in ether and petroleum ether, and the impure crystals from these extractions turned brown on exposure to air at temperatures around 50°C. The yellow compound reacted easily with hydroxylamine to form crystals, which in the impure state melted at 119°C. (uncor.). Attempts to form 2-5 dianilinoquinone resulted in the production of a few red crystals, too scant in amount to obtain a melting point. Quinone titration with potassium iodide, sulphuric acid, and sodium thiosulphate showed the presence of a potassium iodide reducing substance in amounts

equivalent to approximately 0.0006 gm. of quinone per cubic centimeter of solution.

Spectrum analysis of a known solution of quinone in petroleum ether showed four absorption lines at 4960 Å, 4710 Å, 4575 Å, and 4360 Å which correspond to those given for quinone in hexane in the International Critical Tables. Similar analysis of a petroleum ether extract of an oxidized solution of amidopyrine showed two absorption lines occurring at 4673 Å and at 4422 Å in one sample, and at 4660 Å and at 4420 Å in a second sample. The darkest band in each case corresponded approximately with the darkest band in the known quinone solution.

A solution of James' tablets, a proprietary preparation which had been taken in large quantities by one of our patients, the formula of which could not be obtained, gave results identical with the above and indicated, therefore, that the essential component was amidopyrine.

The evidence given above, although not absolute, is strongly indicative that the yellow product formed by the mild oxidation of amidopyrine in weak acid solution and phenacetine in acid and alkaline solutions is quinone. The fact that amidopyrine oxidises much more easily than phenacetine probably accounts for the occurrence of the majority of cases of granulopenia resulting from amidopyrine administration, and the relatively few cases following phenacetine administration.

THE EFFECT OF OXIDATION PRODUCTS IN RABBITS

In a previous communication we have pointed out that benzene when given in small doses is capable of producing granulopenia in rabbits. We further pointed out that this action probably is not due to benzene as such, but to one of its oxidation products, and we have shown that such products as catechol can be recovered from the site of injection and from the bone marrow, whereas benzene is practically absent.

Three rabbits were injected twice daily with 1 cc. of a 25 per cent solution of paraaminophenol-hydrochloride in 5 cc. of normal salt solution for seventeen days, producing no effect on the leukocyte count.

A series of seventeen rabbits were injected with quinone. Eleven of these animals died on the first, second or third day from the immediate effects of the substance. Of the remaining six, two animals showed a complete granulopenia following the daily subcutaneous injections of quinone, one receiving 15 mgm. and the other 100 mgm. The third animal showed a marked depression of the

granulocyte count, whereas no effect was observed in the other three. In working with quinone we have noted that this is an extremely irritating and toxic substance and the chief experimental problem in the production of granulo-

TABLE 5

THE EFFECT OF DAILY SUBCUTANEOUS INJECTIONS OF QUINONE IN OLIVE OIL ON THE LEUKOCYTE COUNT OF RABBITS

100 MG. DOSES		15 MG. DOSES	
Date	Leukocytes	Date	Leukocytes
<i>1934</i>		<i>1933</i>	
3/27	4,950	11/13	12,600
3/28	16,150	11/14	13,850
3/29	9,100	11/15	8,650
3/30	5,500	11/16	10,950
3/31	5,850	11/17	9,200
4/1	2,250	11/18	8,900
4/2	4,200	11/19	5,950
4/3	5,350	11/20	7,950
4/4	3,300	11/21	7,200
4/5	1,550	11/22	5,900
4/6	2,050	11/23	2,850
4/7	1,900	11/24	4,050
4/8	1,750	11/25	6,900
4/9	2,400	11/26	7,450
4/10	2,950	11/27	6,300
4/11*	2,000	11/28	6,900
		11/29	7,500
		11/30	12,750
		12/1	6,000
		12/2	15,850
		12/3	4,050
		12/4	4,650
		12/5	3,950
		12/6	2,550
		12/10**	750

* Animal died with complete granulopenia.

** Animal died with complete granulopenia. In a series of six animals, three showed a marked granulopenia while three others showed little effect on the leukocyte counts.

penia appears to be the regulation of dosage in the individual animal so that an adequate amount is given for the production of granulopenia and yet the dosage remains sufficiently small so as not to kill the animal from its immediate effects.

Thus, we have noted that one animal may be able to tolerate a total dosage of 100 mgm. daily producing granulocytic depression, whereas the same dose would be fatal for another. Table 5 illustrates the effect of this drug on the leukocyte count.

Two animals were injected daily with 5 grains each of the following drugs for sixty days: allonal, barbital, phenacetine, acetanilid, amytal, aspirin and amidopyrine. All drugs were prepared by emulsifying with acacia and water and all

TABLE 6

THE EFFECT OF CATECHOL ON THE LEUKOCYTE COUNT OF RABBITS
Rabbit given daily intraperitoneal injections of 15 mgm. of catechol in 5 cc. of normal salt solution

DATE	LEUKOCYTES
1933	
11/13	6,350
11/14	9,850
	12,150
11/15	10,900
	12,950
11/16	10,800
	13,350
11/17	11,700
	13,900
11/18	5,450
	6,000
11/19	4,900
11/20	3,550
	3,200
11/21*	1,100

* Death. Two other rabbits showed a marked granulopenia while in three others the leukocyte count was little affected.

injections were intraperitoneal. In this series of fourteen animals we observed no depressant effect on the leukocyte count.

Three rabbits were injected daily with 1 cc. of a 25 per cent catechol solution producing granulopenia in two animals with 2000 cells per emm. on the sixth and ninth days respectively. One animal was injected daily intraperitoneally with 15 mgm. of catechol in 5 cc. of normal salt solution producing complete granulopenia on the ninth day with death of the animal. Three other rabbits were injected subcutaneously with 15 mgm. of catechol daily, producing no depressant effect on the leukocyte count. Table 6 illustrates the effect of catechol in one animal in which granulopenia was produced.

In connection with our studies in granulopenia we wish to acknowledge our

indebtedness to the following members of the Emory University faculty whose help has been invaluable: Dr. Joseph Seifter of the Department of Pharmacology, Dr. O. R. Quayle, Department of Organic Chemistry, and Dr. Harris Purks, Department of Physics.

It appears to us that our failure to produce granulopenia by the injection of drugs as such, does not invalidate this conception of the etiology of granulopenia. It is logical to assume that rabbits as well as human beings have an adequate protective mechanism against the action of drugs of this class. This can be illustrated in the animal series of Madison and Squier in which only one of nineteen animals in which the dosage of allonal was so increased that in all probability the protective mechanism was over-whelmed, developed granulopenia. It can be readily seen that the injection of these drugs, whether it be subcutaneous, intraperitoneal or intravenous, would result in little opportunity for oxidation to the more toxic products, whereas the oral administration of the same drugs would, as we have already pointed out, lend them to easy oxidation in the gastro-intestinal tract. It will be noted that oxidation of amidopyrine was produced only in an acid medium. The reaction of tissues at the sites of injections are slightly alkaline. This probably accounts for our failure and that of other investigators to reproduce the condition by injecting this class of drugs. It appears that conditions necessary for easy oxidation in both the experimental animal and the human would be necessary for the production of granulopenia. As to what these conditions are is a problem that remains to be worked out in the future.

SUMMARY AND CONCLUSIONS

It seems that there is constantly increasing evidence that acute fulminant granulopenia and probably the chronic types as well are caused for the most part by the administration of easily oxidizable benzene ring drugs. In the literature there has now accumulated reports of fifty cases in which these drugs are incriminated; notably amidopyrine, occasionally phenacetine and in one instance dinitrophenol. These drugs have been taken with and without barbiturates of various types. So it appears

that barbiturates as a class have little or no effect in the production of the leukopenic state.

The evidence incriminating drugs in the production of this disease has become woven together in a strong chain of circumstantial evidence based on the history of the disease and the coincidental introduction of most of these drugs; based also on statistical data which clearly shows the prevalence of the disease among people of the medical group, obtained from a study of 1314 deaths in this country in the last three years; based also on the prevalence of the disease in Germany and the United States where these drugs have their widest use. We have been able to show that all of the cases of granulopenia under our observation have had drugs of this class prior to the clinical onset. Our studies in the oxidation reactions and the effect of oxidation products in animals indicate that quinone and perhaps catechol are the direct responsible agents in the production of this disease.

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DIAGNOSTIC METHODS IN AMEBIASIS*

RELATIVE VALUE OF STOOL CULTURE AS COMPARED WITH OTHER METHODS

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The widespread occurrence of amebic dysentery and chronic amebiasis throughout the United States has aroused an intense interest in the disease among the members of the medical profession. Despite the fact that many surveys in the past have repeatedly pointed out the incidence of persons harboring *E. histolytica*, in one form or another, the extent of this disease in this country was not fully realized until quite recently, following the discovery of many cases having their origin in Chicago. The important and seriousness of this problem is vividly portrayed in the reviews of recent case reports which have shown the mortality rate in many instances to be extremely high.

Previous investigators have reported several outbreaks of amebic dysentery originating from a single case which was previously unsuspected and undiagnosed. Usually, the originating focus is found to be an "asymptomatic carrier" engaged as a food handler. Recognition, isolation and eradication of this disease from all infected individuals is the only means of combating this problem at the present time. This can be accomplished by a concerted effort on the part of all diagnosticians to render themselves more proficient in the available methods of examination.

Since the solution of the problem depends so much upon proper diagnosis of the condition, a comparative study of the usual diag-

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nostic methods was undertaken. Over a long period of time, various methods have been tried in the different types of cases and statistics kept on examinations of nearly 600 different specimens, including protoscopic aspirations, contents of liver abscesses, et cetera, from as many individuals. Far more examinations than here represented have been made, but comparative data in one phase or another have been kept in this series alone.

Clark² presented a series of 186 cases of amebiasis in which postmortem examinations showed the distribution of amebic lesions in the colon to be as follows: ulcers scattered throughout the colon, 60.7 per cent; ulcers only in certain regions, as cecum, ascending colon, sigmoid and rectum, 33.8 per cent; no definite intestinal ulcers, but scars and amebic lesions elsewhere in the body, 5.3 per cent.

The regions of the large bowel in their order of frequency of involvement, in both the acute and chronic cases, were found to be: cecum, ascending colon, rectum, sigmoid, and appendix. The transverse colon was rarely found to be involved.

Endamoeba histolytica, as its name implies, has definite cytolytic and hemolytic⁵ properties which enable it to attack and successfully invade apparently healthy tissue. The lesions produced usually extend down through the muscularis mucosae and quite often penetrate through the wall of the intestine to the serosa. Usually the ulcer consists of a central necrotic core, surrounded by a reaction zone consisting of lymphocytes, plasma cells, endothelial cells and in the absence of marked secondary infection, relatively few polymorphonuclear leucocytes. Quite a few amebas can usually be seen in the necrotic core, many being easily demonstrable in the wall of the ulcer. Particularly, do the parasites collect around the walls of the necrotic vessels and not infrequently can be seen inside the lumen of the venules. This fact accounts for the frequent occurrence of complicating liver abscesses. These pathologic lesions are usually present in those cases having acute dysentery, the amebas being found in the stools and ulcers in the actively motile, vegetative stage.

In those cases which are usually referred to as chronic amebiasis

or so-called "asymptomatic carriers" the lesions simulate the above, but are very much less extensive; the degenerative and regenerative processes of the mucosal cells being in equilibrium, little or no dysenteric symptoms are produced. The stools of these chronic cases are usually formed and contain the resting or encysted stage of the *E. histolytica*. It is a curious fact and an experience which is not uncommon, that a specimen obtained one day will reveal relatively large numbers of cysts, while on succeeding days, only a few or maybe only one or two degenerated cysts can be demonstrated. Several days or even a week or two later, the stool will contain innumerable cysts again. These cycles of encystation, so to speak, must be borne in mind when examining stools from persons with either chronic or suspected amebiasis. At least two stools, collected at weekly intervals, should be examined in each case before the condition may be ruled out with a fair degree of assurance. Variability of clinical symptoms which these chronic cases present, many cases being completely asymptomatic, makes it impossible to determine the presence of infection from the clinical history or physical examination alone. Thus, the value of routine stool examination in all cases is self-evident.

Descriptions and differential diagnostic criteria of the various types and developmental stages of the pathogenic and non-pathogenic amebas may be found beautifully illustrated in any good textbook on parasitology or tropical medicine.

METHODS OF EXAMINATION

The cases have been divided into two large groups: (1) those cases having acute dysenteric symptoms in which active, motile, vegetative amebas were demonstrable in the ulcers of the bowel or in the liquid stools; (2) those cases not having acute symptoms, a number of which were asymptomatic, having no demonstrable ulcers in the lower bowel and rather formed stools which contained amebas in the encysted stage.

1. Methods of examination in cases of amebic dysentery

The examination of patients having acute dysenteric symptoms of amebiasis should consist of both proctoscopic examination of the rectum and lower sigmoid and complete stool examination, including the presence of blood, Charcot-Leyden crystals and flagellates.

A properly conducted proctoscopic examination will yield a great deal of information besides enabling one to examine directly the contents of the ulcers. In the majority of cases, no preparation of the patient is necessary. However, in some cases, a low enema, consisting of 500 cc. of normal saline solution may be given, allowing about fifteen to thirty minutes for the patient to expel it. Soap suds enemas, besides destroying many of the amebas in the bowel lumen, are irritating and further promote the already existing colonic spasm in the lower bowel which interferes with proper examination. The majority of cases will show considerable involvement of the lower part of the rectum; particularly, the plica transversalis recti, often referred to as Houston's valves. By using a small curet equipped with a long handle or a long piece of glass tubing (2-3 mm. bore), equipped with a rubber suction bulb, the entire contents of the ulcer wall may be obtained. The use of long cotton-tipped applicators usually does not give the best results. Diluting the material obtained from the ulcers with warm normal saline or Ringer's solution is advantageous in making a thin preparation. Direct examination on a glass slide under a cover slip, using a 16 mm. objective, will usually reveal large numbers of the organisms as irregularly quadrilateral, hyaline objects. Under higher magnification, the characteristic morphology and motility is usually easily demonstrable.

Diagnosis from direct stool examination in these cases when properly conducted is not difficult. A stool specimen examined at room temperature will reveal the amebas actively motile for thirty to forty minutes after passage from the bowel. The popular conception that the organisms will immediately cease their motility unless kept at body temperature is erroneous. This has led to the practice of placing the stool in warm water while being transported to the laboratory. It is not only unnecessary, but should the temperature of the specimen be raised above 43° to 47°C. the amebas will cease their motility, the high temperature and dehydration being quite detrimental. A search for amebas in the mucus and blood-streaked particles which come directly from the ulcers will usually reveal them in large numbers, except in cases which have received treatment in one form or another. Of particular note is the observation that bismuth, orally, will render the finding of *E. histolytica* in the stools quite difficult. The bismuth is usually present as the sulphide and identified under the microscope as black, irregularly rectangular crystals.

The presence of Charcot-Leyden crystals and intestinal flagellates with *E. histolytica* in stool specimens from acute amebic dysentery cases, has been pointed out many times before. We have demonstrated these in the majority of our cases.

Some staining methods for vegetative amebas require special technique and a great deal of time. Besides, a study of the motility of the amebas, which is so helpful in diagnosis, is not possible with these methods. For these reasons, although staining by iron-hematoxylin and alcoholic methylene blue methods are valuable for studying the nuclear structure, the simplicity of direct examina-

tion of unstained smears from freshly collected specimens makes this method far more practical.

2. *Methods of examination in cases of subacute and chronic amebiasis*

In this type of case it is usually the cystic stage of *E. histolytica* which is sought. It is an extreme rarity to find the encysted and vegetative stages in the same stool specimen. Sometimes, it is possible to demonstrate vegetative amebas in the bits of mucus which adhere to the surface of a semi-solid stool. However, the cysts which develop from the trophozoites in the lesions high in the colon, are usually found well mixed in the substance of the formed specimens.

The cysts of *E. histolytica* are more easily found in unstained, than in stained preparations. Unstained, under low magnification, they appear as tiny, practically colorless, hyaline, refractile, spheric bodies, easily distinguishable from the debris. Quite often they simulate tiny oil droplets, but are distinguished by their internal structure and staining characteristics. Some observers describe a greenish cast to cysts as seen under low power which aids in finding them on a slide. Under high magnification, the size, shape, thickness of the cyst wall, presence or absence of chromatoid bodies and other distinguishing characteristics, may be studied. However, detailed study of the number and structure of the nuclei requires staining by iodine, iron-hematoxylin or methylene blue.

By emulsifying a small bit of formed stool, the size of a pea, with a drop of water on a slide, it is possible, in heavily infested specimens, to find the cysts. However, concentration methods by centrifugation are easily performed in a few minutes and give a greater percentage of positive results. The simple dilution and concentration method¹⁰ will give satisfactory results if the process of centrifugation is properly conducted. The cysts are well preserved and stain easily and subsequent cultures of these from the sediment is usually successful. This process also removes most of the bacteria and blastocystis, which overgrow the amebas in culture.

The method of Yorke and Adams¹⁵ is useful in obtaining a good concentration of cysts, washing away most of the blastocystis and other bacteria. However, the method is more time consuming than the above and requires special glucose solutions. Primary cultures made from cysts concentrated by this method contains less contaminating bacterial growth, resulting in a more luxuriant primary culture of the amebas.

A method by which cysts may best be concentrated but one which we have found to be more especially adapted to concentration of the ova of intestinal parasites, has been recommended by De Rivas⁷. However, the acetic acid and ether so distort the morphology of the cysts that they appear granular, and the nuclear details are almost indistinguishable when stained. It apparently kills the majority of the cysts as successful cultures from the sediments are very difficult to obtain.

STAINING METHOD FOR CYSTS

As said above, a study of the nuclear structure of cysts requires special staining. By mixing the sediment containing the cysts with an equal part of Gram's or better, Lugol's iodine solution, a satisfactory staining of the nuclei is obtained. Thus, the presence or absence of glycogen bodies, the number of nuclei and situation of the karyosome, all practical distinguishing characteristics, is easily determinable. The technique of this stain is simple and the staining solutions are always available in every laboratory.

McDaniels¹² recently recommended a saturated solution of methylene blue in methyl alcohol as a stain for vegetative and encysted amebas. Although the method gives fair results, we have found that it had no advantage over the iodine stain.

The iron-hematoxylin technique for staining cysts gives excellent results and detailed study of cysts is made possible. However, the special solutions, special technique and length of time required by the procedure, makes this method more adaptable to research than to practical diagnosis. It was also found that the number of cysts seen after completion of the stain, was far less than the number seen in comparative preparations unstained. Apparently, in the process of fixing and staining the wet smears, many of the cysts are mechanically washed off the slide. We have encountered only exceptional cases which required staining by the iron-hematoxylin technique in order to determine the type of organisms present.

CULTURAL METHODS

Boeck and Drbohlav¹ introduced the egg-serum-Locke's medium, consisting of a coagulated egg base superimposed by inactivated blood serum diluted with Locke's solution. This medium gave uniformly satisfactory results and successfully demonstrated the fact that *E. histolytica* was not an obligatory tissue parasite. The addition of acriflavine and rice starch* to the superimposed serum-Locke's or serum-Ringer's solution, introduced by Dobell and Laidlaw,⁸ was a distinct advantage. Craig⁴ then introduced a simple Locke's-serum medium which proved useful for perpetuating an already existing culture of trophozoites. He also demonstrated the fact that a base is not absolutely essential to propagation of *E. histolytica*. In an effort to retain the simplicity of the Locke's serum medium and add to its efficiency, St. John¹³ introduced a similar medium composed of heart muscle extract and Locke's solution to which wheat flour was added. Other modifications have been reported from time to time which have proved of value. Recently, a liver infusion agar-serum-Ringer's-rice starch combination medium was described by Cleveland and Collier.² This medium still retains the essential principles embodied in the original Boeck and Drbohlav medium, the liver infusion agar is substituted for

* Rice starch prepared by Eli Lilly & Co. (Lilly's authentic starches).

the egg base, the superimposed liquid being composed of Locke's-serum-rice starch.

After a preliminary survey, the following cultural methods were selected and an endeavor made to determine their comparative simplicity and initial cost of preparation, practicability and efficacy, as regards positive results during routine examination for vegetative and encysted amebas: (a) Egg-serum-Locke's medium of Boeck-Drbohlav, (b) Egg-serum-Ringer's medium of Boeck-Drbohlav, (c) Egg-serum-Ringer's-rice starch, acriflavine medium of Dobell-Laidlaw, (d) Loeffler's-serum-Ringer's-rice starch-acriflavine medium of Dobell-Laidlaw, (e) Heart muscle-Locke's-wheat flour medium of St. John and (f) Liver infusion-agar-serum-Ringer's-rice starch of Cleveland-Collier.

Method of inoculating and examining cultures. From the cases of acute amebic dysentery, fresh stool specimens or material from the ulcers through a proctoscope or both, were obtained; one or two drops being immediately inoculated directly into two tubes of each of the culture mediums listed above. Only two tubes were used in order to determine the practicability of the method. Certainly, should more than two tubes be inoculated, the chances of obtaining positive cultures are greater, but the practicability of the method is thereby reduced.

At twenty-four to thirty-six hour intervals, the material from the surface of the base, or in the case of St. John's medium, the sediment at the bottom of the tube, was skimmed off with a capillary pipette equipped with a Wright's rubber bulb. The pipette had a large lumen (1 mm.) and its end was broken off squarely after scratching with a small file. The drop of material was placed on a slide, cover glass applied and examination made with the 16 mm. objective. The growth of amebas reached their maximum in twenty-four to forty-eight hours, varying slightly according to the culture medium used (see fig. 1).

The formed stool specimens from routine cases and those suspected of having chronic amebiasis were handled in a similar manner at first. It was soon evident, however, that better results were obtained when the specimen was diluted and concentrated before planting. The washing of the specimen removes the bulk of the bacteria and the majority of the blastocystis, besides concentrating the cysts. A large drop of this washed and concentrated sediment was inoculated into two tubes of each of the medium used. Examination of the cultures was performed as above. The maximum growth of the amebas when specimens containing cysts were inoculated was approximately forty-eight to ninety-six hours (see fig. 2).

Examination of the cultures as described above is not difficult. The amebas retain their motility for approximately one hour at room temperature after removal from the incubator.

The number of trophozoites in a slide preparation from a primary culture of cysts varies considerably. In general, the more cysts inoculated into the medium the more trophozoites are obtained, but exceptions to this are frequently en-

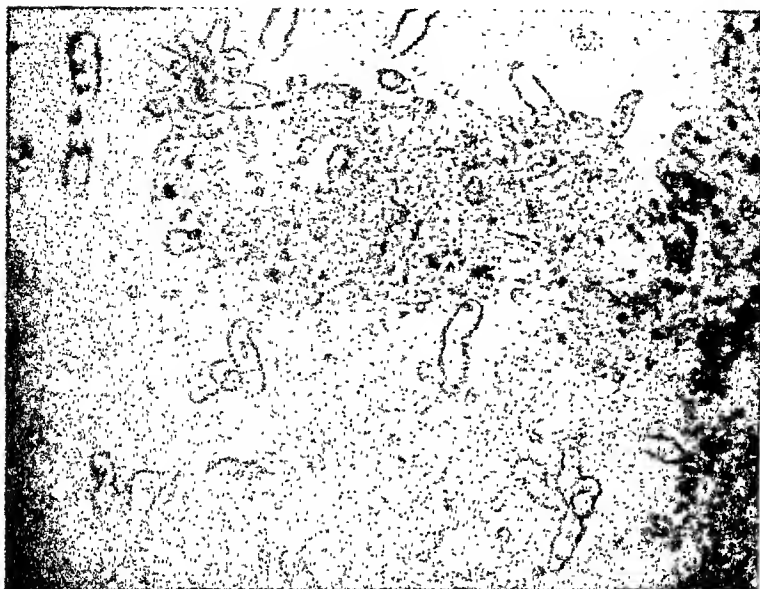


FIG. 1. AVERAGE FIELD SEEN IN SLIDE PREPARATION

E. histolytica trophozoites from twenty-four to forty-eight hour primary culture of vegetative forms from rectal ulcers of acute case of amebic dysentery.

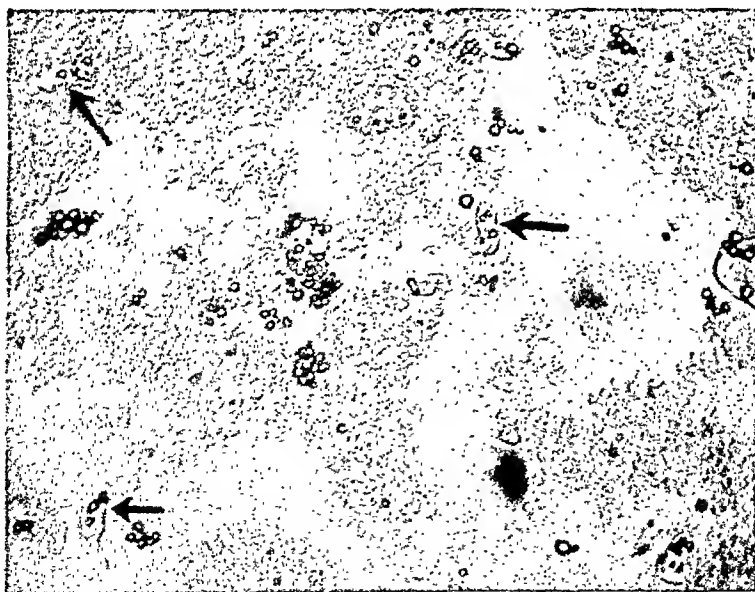


FIG. 2. AVERAGE FIELD SEEN IN SLIDE PREPARATION

E. histolytica trophozoites (arrows) from forty-eight hour primary culture of stool specimen which contained many cysts from "carrier" of amebiasis.

TABLE 1

EFFICACY OF THE MORE COMMONLY USED CULTURE MEDIUMS AS COMPARED WITH
SIMPLE SMEAR AND CENTRIFUGE CONCENTRATION METHODS IN
SPECIFIC CASES*

NUMBER OF CASES	DOBELL- LAIDLAW MODIFICA- TION OF BOECK- DRBOHLAY, EGG BASE	DOBELL- LAIDLAW MODIFICA- TION OF BOECK- DRBOHLAY, LOEFF- LER'S BASE	ST. JOHN'S HEART MUSCLE LOCKE- SERUM WHEAT- FLOUR NO BASE	LEVELAND-COLLIER MODIFICATION OF BOECK-DRBOHLAY LIVER-AGAR BASE	REMARKS
Suspected amebiasis, negative for <i>E. histolytica</i> by direct examination					
7	-	-	-	-	Cases of previously proved and treated amebiasis.
5	-	-	-	-	Only cysts, morphologically <i>E. coli</i> and <i>E. nana</i> , present by direct microscopy.
3	-	-	-	-	Cases of chronic bacillary dysentery.
2	+	-	-	-	Only cysts, morphologically <i>E. coli</i> , <i>E. nana</i> and <i>I. buetschlii</i> , present by direct microscopy.
2	+	-	-	+	Only cysts of <i>E. nana</i> present by direct microscopy.
1	+	-	-	-	Case of proved amebiasis. Specimen of 3rd day after specific treatment started. Negative by all methods thereafter.
1	+	-	-	-	Only one atypical cyst in sediment by direct microscopy.
Amebiasis, positive for trophozoites of <i>E. histolytica</i> by direct examination					
1	+	+	-	- (Gas produc- tion due to bacterial growth)	St. John's media 3 mos. old. Cleveland-Collier media unsatisfactory; "blew up" due to gas. Subsequent batches O.K.
4	+	+	+	+	New St. John's and Cleveland-Collier media used.
Amebiasis, positive for cysts of <i>E. histolytica</i> by direct examination					
1	-	-	-	- (Gas, etc.)	Specimen 3 days old before culture.
4	+	+	-	- (Gas, etc.)	
4	+	-	-	- (Gas, etc.)	
1	+	-	-	-	New batch of Cleveland-Collier medium.
4	+	-	-	-	New batch of Cleveland-Collier medium.

* Two tubes of each medium inoculated in a single attempt. From nearly 600 patients, including previous work, 41 specimens containing cysts characteristic of *E. histolytica*, successful cultivation in Dobell-Laidlaw or Cleveland-Collier modification medium was possible in all cases when sufficient cultures (as many as ten) were made from as many as three different specimens.

countered. Sometimes, only one or two trophozoites are seen on an entire slide; other cultures from other cysts may show ten to twenty trophozoites per field of a 16 mm. objective.

Further details of preparation of media, inoculation and examination may be found in a previous report by one of us.¹⁴

RESULTS

A comparison of results obtained in the majority of representative specimens is shown in table 1.

DISCUSSION

Many controversial statements may be found concerning the value of cultural methods as compared with direct examination of stools for *E. histolytica*. Indeed, even as regards the different modifications of culture mediums in use, the opinions vary as regards the efficacy of each one. Some investigators^{3, 6, 9, 11} say that for routine use, it is superior to direct microscopy and others that it is inferior.

Vegetative amebas, either from an untreated acute case or previous culture, when inoculated into any of the mediums used in this series, will be propagated indefinitely under proper care. The Dobell-Laidlaw and Cleveland-Collier Mediums give the most luxuriant growth on primary culture. However, St. John's medium, although less efficient as regards primary culture and luxuriance of growth, has the advantage of simplicity of preparation and less bacterial growth. The carbohydrate of the large particles of wheat flour is not easily available and consequently growth of blastocystis is inhibited to a remarkable degree. This medium is most useful in propagating stock cultures of amebas.

The morphology of the trophozoites of *E. histolytica* as seen in culture is not remarkably changed from those seen directly in the stool or from the ulcers. Of course, erythrocytes are no longer present in the endoplasm, but their place is taken by starch granules from which the organisms derive their food supply. The amebas grown on St. John's medium are smaller in size than those from the other mediums, and their motility is less characteristic, but the other morphological characteristics are still retained.

The Cleveland-Collier medium is a little more costly to prepare than the others. Some batches of it have proved totally unsatisfactory, due to the fact that bacterial action upon the base produced gas, which caused the whole tube of media to "blow up," so to speak. In a few instances, the base was almost completely extruded from the tube, resulting in loss of the culture. However, other batches have not shown this same phenomenon and have proved satisfactory. Possibly, aging of the ingredients was responsible although we purchased the material fresh and used it in a short time. Perhaps, some change in the preparation of the ingredients during manufacture may account for the unsatisfactory results at times.

The Dobell-Laidlaw modification is cheaply prepared and all ingredients are readily available, no purchase of materials or time for delivery being necessary. The rice starch may be kept indefinitely. This medium has proved to be superior to the others used in this work and may well be advocated as a practical, efficient diagnostic aid in routine work.

Initial primary cultures from *E. histolytica* cysts are not as uniformly successful as with the trophozoites. Cysts in specimens not over twelve to twenty-four hours old result in more successful cultures than older cysts. Several specimens contained cysts which failed to excyst in the initial culture in the two tubes of one or the other of the mediums. We believe that should sufficient tubes be inoculated, positive cultures will be obtained from all fresh specimens containing cysts.

The cultural methods used are practically specific for *E. histolytica*. This is an advantageous feature of the cultural method in identifying cysts. The other amebas usually do not excyst and grow. In a few instances, *E. coli* and *E. nana* trophozoites may be found. They retain their morphological characteristics but, unlike *E. histolytica*, do not engulf starch. On subculture, the *E. coli* and *E. nana* trophozoites die out; in contrast to *E. histolytica*, which becomes more prolific with each subculture. In some of the cultures of *E. histolytica* which have been transplanted many times, the growth is so luxuriant that slide preparations show every field of the microscope literally crowded with amebas.

The stool specimens from two cases contained cysts morphologically like *E. coli*, *E. nana* and *Iodamoeba buetschlii*, as identified by direct examination. However, on culture typical trophozoites of *E. histolytica* were found and confirmed by subculture through many generations. Apparently, a few *E. histolytica* cysts were present, but not identified by direct examination.

In one case by direct examination, only one cyst could be found in the entire examination. The identification in such cases is extremely difficult. Careful study and staining may be practically impossible for a very few cysts. Cultivation of the sediment will usually add a great deal of information for identification of species.

Some small strains of *E. coli* cysts and some large strains of *E. nana* cysts in feces may so closely simulate unstained *E. histolytica* cysts morphologically that positive identification without staining is difficult.

The vegetative stage of *E. coli* in cancerous or syphilitic ulcers of the rectum may simulate the trophozoites of *E. histolytica* in that they may engulf red blood cells under these conditions. An error in diagnosis may be avoided if subsequent studies of the organisms in culture be performed.

The study of the vegetative forms and cultural characteristics of the three intestinal amebas more commonly encountered in practice, in addition to the study of the encysted stage, gives sufficient information to identify the organisms in the majority of cases. These studies at least give as much information and is less time-consuming and more practical than the study of iron-hematoxylin stains.

The cultural methods are practical and may be used in the average laboratory without any additional cost. The technique is not any more difficult than that required for routine bacteriological work. In general, the results of a worker just beginning to use cultural methods in diagnosis are usually not remarkably inferior to one who has been using the method for a long time. The technique is easily mastered in a short time. Certainly, as compared with learning special techniques, such as iron-hematoxylin and differential criteria of stained cysts, the method is simpler, less time-consuming and cheaper.

The complement fixation test of Craig⁵ for the diagnosis of amebiasis has not been sufficiently developed to render it available for routine use. The results obtained are not conclusive enough to make it a reliable method as yet. However, improvements are constantly being made and the future possibilities of the test are encouraging.

The use of alcohol-glycerin-saline extracts of luxuriant cultures of *E. histolytica* as an antigen for skin tests has been used by one of us (C. J. T.). Extraction of all the bacteria also occurs and consequently non-specific reactions interfere with proper interpretation of the results. Until amebas can be grown in pure cultures in the absence of all bacteria, satisfactory extracts for skin testing will be difficult to obtain.

SUMMARY AND CONCLUSIONS

(1) A comparative study of the various diagnostic methods used in acute and chronic amebiasis is presented.

(2) Stool specimens and contents from rectal ulcers of acute cases have been studied for *E. histolytica* trophozoites in fresh unstained smears, iodine, methylene blue and iron-hematoxylin stained smears and by various cultural methods.

(3) In acute cases, examination of fresh unstained smears and subsequent cultures of the material have been found to be the most practical and efficient procedures.

(4) In chronic or suspicious cases, stool specimens were examined for cysts by direct smears, concentration methods, iodine, methylene blue and iron-hematoxylin stains and cultural studies.

(5) A most practical and efficient procedure of examining stools for cysts is the simple water dilution and centrifuge method. Staining the cysts with Lugol's iodine solution will satisfactorily reveal the nuclei. Subsequent culture permits study of the trophozoite stage of *E. histolytica*.

(6) The Dobell-Laidlaw modification of the Boeck-Drbohlav medium was found to be the most practical and efficient cultural method in routine examination of material for trophozoites and cysts of *E. histolytica*.

(7) A study of the vegetative forms and cultural characteristics

of the three more common intestinal amebas, in addition to a study of the encysted stage, gives sufficient information for practical identification in the majority of cases.

(8) The cultural methods for *E. histolytica* are practical and may be used in the average laboratory without any additional cost or special training.

We wish to thank Mr. C. C. Adams of this department for his valuable technical assistance throughout this work.

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RAPID PIGMENT APPEARANCE IN OHIO RED BELLIED DACE AS TEST FOR INTERMEDIN*

PRELIMINARY REPORT

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Zondek and Krohn⁵ in 1932 demonstrated conclusively that the hormone regulating pigment metabolism is elaborated by the pars intermedia of the hypophysis. Furthermore, they found a small fish native to Germany (the elritza) an ideal test object for the demonstration of the effect of the hormone since the adult male with silvery undersurface (except in the spawning season) when injected with a small amount of an extract of the intermediate lobe of the hypophysis quickly develops a specific brilliant red coloration. They named the hormone intermedin and found no excess of the hormone in the blood or urine of man in health or in disease.

After a year's unsuccessful attempts to secure a supply of elritza from Germany, a search was made of the literature of domestic fish. Impressed by the similarity of the red bellied Minnow (*Chrosomus erythrogaster*) of Ohio (Jordon³) with the elritza, a supply of the former was obtained in March 1934. It was found that this minnow, also known as the Ohio red bellied dace, reacts beautifully after the injection of material containing intermedin, the reaction appearing within one half hour and persisting for four to twenty-four hours.

The first important work relating to pigment metabolism was that of Hogben and Winton¹ who reported on the action of posterior pituitary extracts on the melanophores of frogs. They stated that the hormone controlling pigment metabolism was most abundant in the intermediate lobe. The results of Hogben

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

and Winton in frogs were confirmed by Allen and Atwell but it remained for Zondek and Krohn to introduce the ideal test animal. Since the work of Zondek and Krohn, Ferguson² (1933) using imported elritza as the test object found a demonstrable amount of intermedin in the blood of patients having melanosarcoma and neurogenic sarcoma.

The red bellied minnow measures 5 to 8 cm. in length (same size as elritza). In the spring the males have a bright red belly. Their habitat is the clear brooks from the Ohio valley to the Red River of the North. They differ from the elritza in having a rounded rather than a flat body. The upper surface of the body of both male and female is of an olive drab color. The fish when first isolated from the stream require an environment simulating that of their natural habitat as closely as possible, but may be gradually adapted to the conditions of the ordinary aquarium and as supplied by the dealer thrive on prepared foods. The most essential requirement is the maintenance of live water plants in the aquarium to absorb harmful substances, such as chlorine. Under proper conditions the fish may be bred in the aquarium, the local dealer depending almost entirely upon this source of supply.*

As stated above, the male dace reacts beautifully to intermedin. During the spawning season, May and June, when the water is warm (about 20°C.) the male fish spontaneously develops a flame colored pigmentation of the under surface of the body. By keeping the water in the aquarium cold (about 15°C.) the pigmentation disappears and with properly controlled temperatures the fish may be used as test objects during this season.

Saphir⁴ has reported on "Artificial Production of 'the Wedding Dress' in *Chrosomus Erythrogaster*" commonly called red bellied dace. He obtained positive results with yohimbine hydrochloride and inconstant results with prolan as isolated from the urine

* Wm. Tricker, Inc., Independence, Ohio and Saddle River, New Jersey. We wish to express our appreciation to Mr. J. L. Charleson, Manager of the Independent Plant, for helpful suggestions as to maintaining the life of the fish in the aquarium.

of pregnant women. He also obtained a positive result with urine extract of a case of chorionepithelioma.

The work presented in this preliminary paper was done with a few hundred red bellied dace obtained since March. A study was made of the behavior of the fish to injection of intermedin, other hormones, and urine of patients in health and with various diseases.

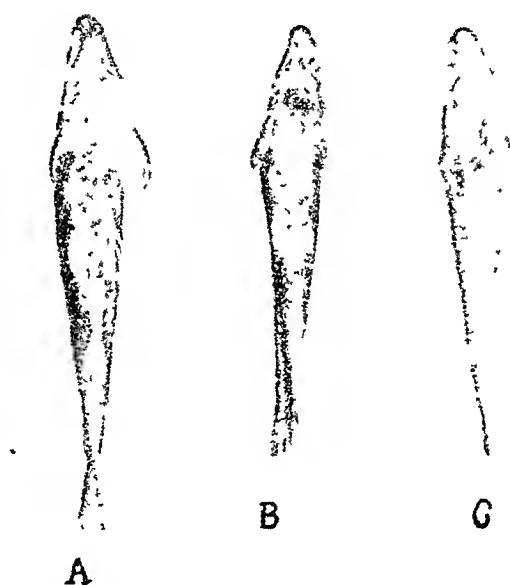


FIG. 1. PHOTOGRAPH OF RED BELLIED DACE

A. Strongly positive result in a case of melanosarcoma of the eyeball. B. Spontaneous pigmentation in the spawning season. C. Negative control. The dark ventral surfaces of A. and B. are actually a flame red color. Ventral surface of C. is actually silvery.

TECHNIC OF THE TEST

In general the technic of the test is as described by Zondek and Krohn⁵ in the elritza. Healthy adult male fish measuring 6.5 to 7.5 cm. in length are selected. The fish are held in a wet cloth in the left hand and a fine hypodermic needle attached to a 1 cc. (tuberculin) syringe is inserted just back of the dorsal fin for a distance of about 1 cm. to avoid leakage of the material injected. The injection is made just beneath the skin to avoid penetrating the air bladder of the fish. The volume of the material injected varies from 0.1 to 0.3 cc., small doses being better tolerated. After injection, the fish are placed in individual

glass bowls for observation. The dace tolerate the necessary manipulations out of water well.

Except in the spawning season, the male fish has a silvery under surface. In a positive test, the under surface develops a brilliant flame red color apparently due to rapid elaboration of pigment as shown by Zondek's⁷ demonstration that four and a half times as much red pigment can be extracted from the deeply pigmented skin as from the unpigmented controls.

Zondek defines one elritza unit of intermedin as the minimum amount of hormone in which three out of five elritza (6.5 to 7.5 cm. long) produces about the

TABLE 1
RESULTS OF TESTS WITH VARIOUS HORMONES IN RED BELLIED DACE

HORMONE	STRONGLY POSITIVE	POSITIVE	DOUBTFUL	NEGATIVE
Posterior pituitary extract	10			
Anterior pituitary extract	8			
Corpus luteum		2		1
Amniotin			2	1
Theelin			1	
Antuitrin S				1
Prolan				1
Androtin		1	3	
Parathormone		1	1	1
Epinephrin				1
Insulin				1
Thyroxin				1
Thyrovorian				1
Adrenal extract				1
Mixed gland (male)				1
Mixed gland (female)				1

mouth, breast, abdomen, and from the anal fin back to the anus, in an area 4 to 9 sq. mm. large, a striking formation of light purple red color. The reaction must begin within one half hour and lasts for four hours.

In the red bellied dace, it has seemed advisable to consider as a doubtful reaction one in which there is an appearance of one or more small areas of pigmentation measuring less than 1 sq. mm. in diameter anywhere on the under-surface. A positive reaction consists in a streak of red pigmentation extending along each side of the body from the buccal fin back to the anal fin. A strongly positive reaction consists in a brilliant pigmentation of the entire under-surface of the body. These changes must appear in two out of three injected fish.

RESULTS

Strongly positive results were obtained with extracts of the posterior lobe of the ox pituitary which also includes intermediate lobe. Extracts of the anterior lobe were also positive probably

TABLE 2
RESULTS OF TESTS FOR INTERMEDIN IN RED BELLIED DACE

DIAGNOSIS	MATERIAL INJECTED	POSITIVE	FALSE POSITIVE	DOUBTFUL	NEGATIVE
	Ringer's solution with 0.25 per cent acetic acid				2
	Physiological saline				3
Melano-sarcoma, eyeball	Urine conc. 5 X	1 (Pre-op.)			1 (Post-op.)
Neurogenic sarcoma	Urine unconc.	1 (Pre-op.)		1 (Post-op.)	3 (Post-op. and post X-ray)
Various clinical conditions other than neurogenic sarcoma and melanosaarcoma	Urine conc. 5 X		2		11
	Unconc. urine		2		27
Hydatid mole					1
Pregnancy					1
Negro					2

due to postmortem diffusion of intermedin into the anterior lobe. Weakly positive results were obtained with corpus luteum extract, amniotin and theelin. Variable results were obtained with androgin and parathormone. Negative results were obtained with the other hormones tested. (See table 1.)

As the pituitary extracts were made up in 0.25 per cent acetic acid, control fish were injected with Ringer's solution containing 0.25 per cent acetic acid and with physiological saline solution. These tests were uniformly negative. In a case of neurogenic sarcoma the urine of which was given us through the courtesy of Dr. F. Bayless of the Institute of Pathology, Western Reserve University, a positive result was obtained preoperatively, a doubtful result postoperatively, and three negative results following X-ray therapy. In a case of melanosarcoma of the eyeball a positive result was obtained five days after removal of the eyeball and a negative result subsequent to removal of the remaining orbital tissue which on microscopic examination showed a small amount of tumor tissue. In a series of thirteen tests in which the urine was concentrated according to the method* described by Zondek⁶ two false positive results were obtained.

In a second series of twenty-nine cases in which unconcentrated urine was injected, two false positive results occurred. Tests done with urine from two negroes were negative. Table 2 shows results of injection of urine in various clinical conditions.

A number of urines, both concentrated and unconcentrated, were toxic and the fish died before sufficient time had elapsed for reading the test.

Additional experiments are under way to determine the specificity of the dace as a test object for the demonstration of intermedin.

CONCLUSIONS

There is a rapid red pigment appearance in the Ohio red bellied dace similar to that in the elritza following injection of extracts and urine containing intermedin.

In the small series of tests so far completed, positive results were also obtained with urine from one case of melanosarcoma and one case of neurogenic sarcoma. Non-specific results with urine,

* Add to the urine to be tested an equal quantity of 95 per cent alcohol and concentrate to one-tenth the volume by evaporation on the water bath thus concentrating the urine five times. (Intermedin, unlike the hormones of the anterior lobe of the pituitary, is not destroyed by boiling.)

however, have occurred in a few cases apparently unrelated to these tumors.

A large series of experiments will be necessary to determine whether or not the positive reaction in the red bellied dace is a reliable index of the presence of intermedin.

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ENDOCARDITIS CAUSED BY DIPHTHEROID BACILLUS (PLEOMORPHIC STREPTOCOCCUS)*

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For many years the tendency of most workers has been to consider diphtheroid bacilli obtained from human sources, especially from blood cultures as contaminants or as mere saprophytes. This view in general is taken by the British Medical Research Council⁵ in their summary of the literature up to 1923. However, some have considered these organisms to be of pathogenic significance. Recently Kessel and Romanoff¹⁰ fairly completely summarized the literature in regard to infections by diphtheroid bacilli and described a non-fatal case of generalized infection, including meningitis, in which four blood cultures and the spinal fluid revealed diphtheroid bacilli.

No case of endocarditis has yet been described in which diphtheroid bacilli have been found alone in the heart vegetations. Tow and Wechsler¹⁶ found in smears of the heart vegetation of a child of eleven who had been sick from endocarditis for six weeks, many diphtheroid organisms and a few Gram positive cocci, singly or in pairs. No cultures of the valve were made. From three cultures of the blood a diphtheroid bacillus which grew abundantly on ordinary media and which had the characteristics of *Corynebacterium hoffmanni* was obtained. It produced methemoglobin in forty-eight hours on blood agar. They believe that the streptococcus found in smears was the etiologic agent and that the diphtheroid bacillus was a secondary invader. Babes and Manolescu² also described a case in which in sections of the

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heart vegetation, diphtheroid bacilli and in addition a few diplococci were found in an undulating layer on the outer surface of the fibrin layer. Howard⁵ reported a case of a man of forty-four who had been sick for seventeen days with endocarditis but no clinical signs of diphtheria, from whom a bacillus morphologically a true Klebs-Loeffler bacillus, showing beaded forms and some clubbed ends were seen in sections and smears. Cultures on simple media were identical with true diphtheria bacilli. The organism failed to kill guinea pigs or rabbits. The bacteriology of this case was checked by Dr. Welch, but at that time no fermentation tests were done. This may possibly have been a diphtheroid organism. Herzog⁷ also described a patient sick for four weeks who died of endocarditis without clinical signs of diphtheria. In the heart vegetations numerous diphtheria bacilli were found in smears and sections. Cultures from this killed a guinea pig in five days. No control animal given antitoxin was used.

Numerous reports are found in the literature concerning the mutation of organisms from diphtheroid to streptococcic forms. Mellon¹¹ and Rosenow¹² in this country have for a long time maintained that changes from the bacillary phase of an organism to the streptococcic phase can be produced by transferring the organism from solid media to proper liquid media. Jensen and Morton⁹ described two strains, one derived from the urine of a case of cystitis and the other from the blood culture of a patient with subacute endocarditis, which they could change from diphtheroids on blood agar to streptococci in dextrose brain broth and back and forth at will. Sinek and Springer¹³ described a typical case of subacute bacterial endocarditis in which a necropsy was performed; smears from the heart valve showed large numbers of streptococci. On culture a markedly hemolytic streptococcus was obtained but after repeated transfers it became similar to cultures obtained from the blood before death; it lost its power of hemolysis and both strains showed typical diphtheroid rods on solid blood-containing mediums and streptococcic chains in liquid mediums. Abercrombie and Scott¹ described a case of a boy of eighteen with congenital heart disease who died of sub-acute

bacterial endocarditis from whom *Streptococcus mutans*, which was non-hemolytic and non-green producing was cultured from the blood before death and from the heart vegetation at necropsy. This organism grew typically in chains of cocci on liquid medium but on glucose agar the majority of the organisms were typical diphtheroid bacilli. On changing to glucose-free medium the culture reverted to the original streptococcal form. These authors state that Clarke who described the organism first from carious teeth had observed three cases of fatal endocarditis caused by the same streptococcus.

Beck and Braun³ described marked changes in the growth characteristics of various strains of streptococci and pneumococci when grown in urine, starting with mixtures of urine and broth and increasing the amount of urine. From all the various strains used, as the final product, enterococci (type B) were obtained, which resisted 60°C. for one hour and had lost their pathogenicity for animals. Fleck and Elster⁶ also reported marked changes in colony structure and morphology in a pigmented strain of streptococcus by changing the medium or by sufficiently repeating transplants. Bacillary forms typical of *Corynebacteria* were obtained on solid media. Straus¹⁴ in studying blood cultures from cases of chronic infectious arthritis obtained diphtheroids in seventy-four cases out of 750 cultures. By Mellon's method he was able to transmute twenty-four out of thirty-six of these cultures to streptococci, but he was unable to revert them back to bacillary forms. The bacillary forms varied from the streptococcal forms derived from them in their fermentation reactions and antigenic properties. Callow¹ found results similar to those of Straus in her extensive work. She cultured bloods from large numbers of cases of rheumatic fever, acute diseases of the respiratory tract, including tonsillitis and measles, miscellaneous febrile and afebrile diseases, and a series of normal persons. Of the rheumatic fever cases 70 per cent had a positive culture. Fifty-one per cent of the positive cultures were pleomorphic bacilli, 35 per cent were *Streptococcus viridans*, both organisms, 7 per cent, and 6 per cent were anhemolytic streptococci. In the series of acute respiratory diseases 70 per cent yielded a positive culture with

about the same proportion of the various organisms as in the rheumatic fever group. In the miscellaneous febrile group 6 per cent were positive, in the miscellaneous afebrile disease group 45 per cent, and in the normal person group 8 per cent. Most of the cultures from the afebrile cases and all the cultures from normal persons were pleomorphic bacilli. These grew with difficulty. Callow was able to transmute the pleomorphic bacilli into streptococci in all cases tried by culturing on potato phosphate broth. Some changed to anhemolytic streptococci, some to viridans type and a few to hemolytic strains. She concluded that "alpha diplostreptococci and pleomorphic bacilli may be recovered repeatedly from the blood of patients with rheumatic fever and certain diseases, mostly of the upper respiratory tract. These organisms apparently represent stages in the life cycle of the same organism. A specific etiological relationship between these organisms and rheumatic fever is questioned."

Thompson¹⁵ in 1079 blood cultures taken in 730 cases of various illnesses at the Mayo Clinic, found 33 cultures of diphtheroids, an incidence of 3 per cent. Only one of the cultures, obtained from a case of subacute bacterial endocarditis, showed mutation to streptococcic forms when transplanted to liquid medium, and this form persisted on further subcultures. The others remained bacillary at all times. He concluded that diphtheroids appear to have the same significance in blood cultures as do saprophytic cocci.

These unique findings warrant special attention to pleomorphic or diphtheroid bacilli and even to streptococci when found in blood cultures. Only by repetition of similar experiments and thorough study of bacteria from cases examined post mortem, especially those of rheumatic and non-rheumatic cases, can the significance of these organisms be determined. The following case report deals with a case in which typical diphtheroid bacilli were found in blood cultures and in the heart valve. It could be mutated partly to streptococcic forms.

CASE REPORT

Mrs. A. H., housewife, age 36, had in her earlier years suffered from a variety of infections, including scarlet fever, influenza, typhoid fever at the age of fifteen,

and repeated attacks of tonsillitis. Her tonsils had been removed at the age of twenty-three. Nine years before her death she suffered an attack of acute rheumatic fever with mitral endocarditis, and had known since then that she had "leakage of the heart." Three years before death her upper teeth were extracted but there were left two roots which from X-ray evidence were not infected.

Eleven months before death she began to lose weight, fatigued easily and became nervous and irritable. In about three months she became bedridden because of increased weakness, slight fever, breathlessness and palpitation on exertion and slight diarrhea. Her attending physician, Dr. C. L. Haines, suspected a possible mild exacerbation of a minimal chronic pulmonary tuberculosis. Two months later she complained of rheumatic pains in her large joints. A blood culture at this time was reported as being negative. During the next two months her condition remained unchanged, and she was brought to the Pasadena Hospital on May 28, 1933 for study. She complained on admission of weakness, nervousness, loss of weight, palpitation, and pains in her shoulders and knees.

Physical examination revealed a moderately pale woman, fairly well nourished, showing no edema or pectechiae in the skin or elsewhere. The thyroid was negative. A moderate enlargement of the heart and a systolic and presystolic murmur was heard plainly at the apex and transmitted to the axilla, typical of a mitral stenosis. The spleen and liver were not palpable, no masses or tenderness were found in the abdomen. The rest of the examination was negative. Her temperature reached 100 to 101° every day, pulse 80 to 90, respiration normal.

Laboratory examinations revealed 11.3 grams of hemoglobin (Newcomer); erythrocytes, 3,510,000; leukocytes, 10,900; segmented neutrophils, 35 per cent; non-segmented neutrophils, 17 per cent; lymphocytes, 23 per cent; eosinophils, 1 per cent; monocytes, 18 per cent, and histiocytes, 6 per cent. Many of the monocytes were immature and showed toxic budding. Many of the histiocytes showed phagocytosis of erythrocytes or nuclear fragments. The erythrocytes showed slight achromia and minimal variation in size. The urine was negative. Two blood cultures planted in infusion broth, brain dextrose (1 per cent) broth and pour infusion plates gave a pure growth of diphtheroid bacilli in all flasks and plates, averaging about 10 to 15 colonies per cc. of blood in the plates. (See below.)

A presumptive diagnosis of bacterial endocarditis was made and after a week she returned to her home. During the next two months her rheumatic pains continued, fever was constantly present, distressing attacks of paroxysmal tachycardia occurred at irregular intervals, associated with vomiting and diarrhea, which necessitated her second admission to the hospital on August 5, 1933.

By this time she had become markedly emaciated and anemic. The spleen was palpable three finger breadths below the ribs. The heart findings were the same as at first admission. A ballotable right kidney was palpable and in the

lower portion of Scarpa's triangle of the left thigh, deeper than the femoral artery, a hard tender, non-pulsating, fixed mass the size of a pigeon's egg was felt. About three weeks previously this mass had been exquisitely tender.

Laboratory studies at this time revealed 8.3 grams of hemoglobin (New-comer); erythrocytes, 2,950,000; leukocytes, 9,700; segmented neutrophils, 73 per cent; stab forms, 15 per cent; juveniles, 1 per cent; monocytes, 5 per cent; lymphocytes, 6 per cent. No histiocytes could be found. Several fresh urine

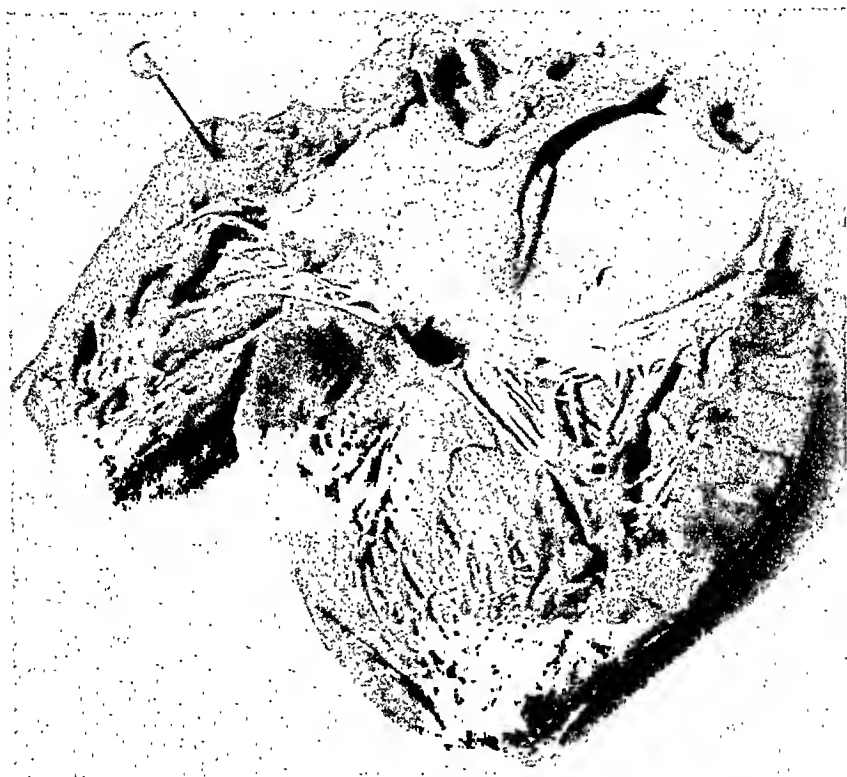


FIG. 1. HEART

Note the vegetations on the mitral valve and the old fibrous thickening of the leaflets and chordae tendinae.

samples were grossly bloody, showed 1+ to 3+ albumin, and a heavy sediment of leukocytes and erythrocytes. No casts were found. Smears and cultures revealed large numbers of hemolytic colon bacilli and streptococci but no diphtheroids. Two blood cultures gave a growth of diphtheroid bacilli similar to those cultured ten weeks previously.

The clinical course was steadily downhill until her death on September 12, 1933. During the last few weeks of life the urinary output decreased, the albumin content was always high, and erythrocytes were more numerous.

Terminally a few moderate sized ecchymotic spots appeared on the left forearm. She was anuric and comatose for the last few days. The final clinical diagnosis was made of subacute diphtheroid bacillus endocarditis, acute glomerulonephritis, acute pyelonephritis, uremia, and mycotic aneurysm of left thigh.

Necropsy was done two hours after death. The final anatomical diagnosis was: subacute recurrent diphtheroid bacillus endocarditis of mitral valve and old fibrous endocarditis of mitral valve with moderate stenosis and insufficiency; recent endocarditis of aortic leaflets; acute diffuse glomerulonephritis, uremic enterocolitis; acute right pyelonephritis and cystitis; completely thrombosed infective (mycotic) aneurysm of left profunda femoris artery; anemic infarcts of various ages in the spleen; marked anemia and emaciation.

The heart (fig. 1) weighed 230 grams (total body weight, about 36 kg.) and measured 11 cm. long and 9.5 cm. wide. The mitral leaflets showed considerable old fibrous thickening, in some places to 4 or 5 mm. thick, and fusion of the leaflets at the angles for about 1 cm. On the opposing margin of the anterior leaflet in its middle was found a bean-shaped vegetation, firm to the feel, measuring about 1.3 x 0.5 x 0.3 cm. and firmly fixed to the valve. The surface was fairly smooth, except at one end, which was somewhat roughened. On section there was marked organization at the point of attachment and an occasional fleck of calcium was found in the middle of the vegetation. Small, fine, firm, partly organized vegetations were found on the posterior leaflet, over its entire middle and extending onto the adjacent auricular endocardium, and at the angle between the two leaflets. There was moderate fibrous thickening, fusion, and some shortening of the chordae tendinae of both leaflets, chiefly the anterior.

A small, firm, gray vegetation 4 mm. across was present in the middle of the posterior leaflet of the aortic valve 5 mm. from its top margin. There was a moderate hypertrophy of the left auricular muscle. The muscle of the ventricles was pale and moderately soft. The coronaries showed no noteworthy changes. There were no congenital defects. The valves of the right side of the heart were normal.

Histological study of hematoxylin and eosin and Gram-Weigert stained sections through the vegetation and the deeper portions of the mitral leaflets (fig. 2) showed marked fibrosis and occasional small calcium deposits in the thickened part of the valve. Just external to this was a layer of granulation tissue showing many fibroblasts, round cells and macrophages, and on the surface the bulk of the vegetation was composed of partly hyalinized fibrin or necrotic material, in the meshes of which large colonies of bacilli were arranged in irregular masses or colonies, chiefly in the deeper layers. Some small colonies or groups of bacilli were found just at the margin of the granulation tissue and a few were found within macrophages. In Gram-Weigert stains the organisms were most plainly seen. They were strongly Gram-positive and were all bacillary in type, much longer on a whole than those obtained on cultures on blood agar, sometimes showing long filamentous forms, chiefly growing in colonies, but some were found

quite separate from these groups. No streptococci or other organisms were seen. Small masses of calcium were present in the necrotic portion of the vegetation. Large masses of poorly staining bacteria were commonly seen, and in some of these, calcium deposit was present.

The changes in the other organs are given sufficiently above. The infection of the pelvis of the right kidney and bladder was fairly recent as was the diffuse glomerulonephritis.

Cultures of the heart blood after death revealed a heavy growth of diphtheroid bacilli similar to those obtained in life. Cultures made aseptically from frag-

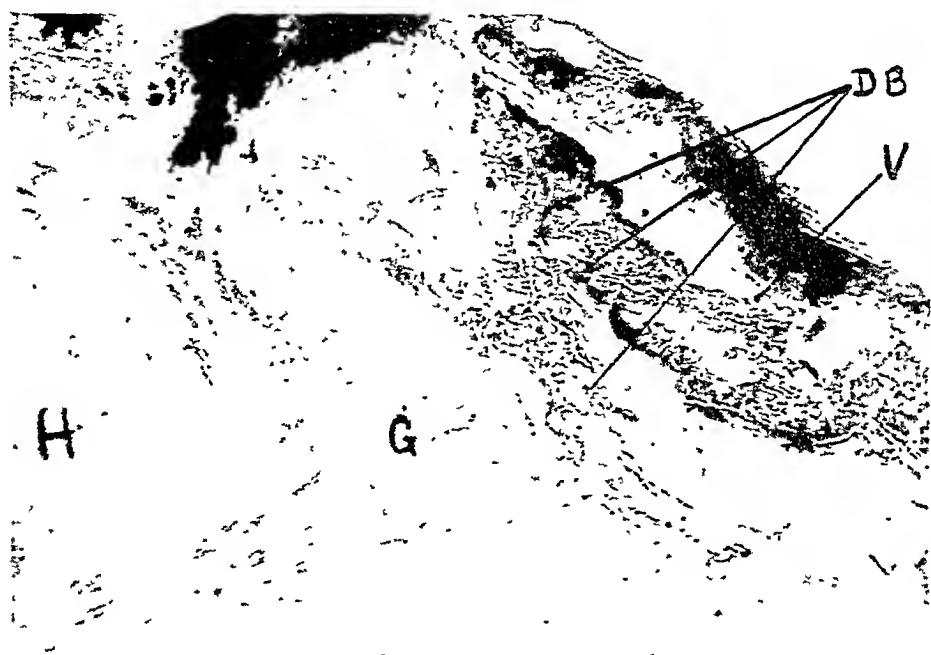


FIG. 2. SECTION THROUGH MITRAL VALVE

This shows masses of diphtheroid bacilli (D. B.) in the vegetation: hyaline fibrous portion (H.) and granulation tissue (G.) just below vegetation (V.).

ments of the heart vegetation yielded a heavy growth of the same organism. Smears of other pieces showed moderate numbers of Gram positive bacilli, chiefly elongated forms, separate or in clusters, of the same diameter as those found in blood agar cultures, but on the average about twice as long, and some long filaments as long as four or five average bacilli. No other organisms were present.

The organism in the original culture of the blood and heart valve in bouillon and blood agar grew slowly, and colonies in blood agar pour plates were about half the size of a pin head at their maximum growth, almond-shaped, and sur-

rounded by a narrow green zone. The surface colonies were round, gray, glistening, minute in size, and likewise showed a narrow green zone, but no hemolysis. The colonies were so much similar to green producing streptococci that we were always surprised on examining smears made from all the colonies that only

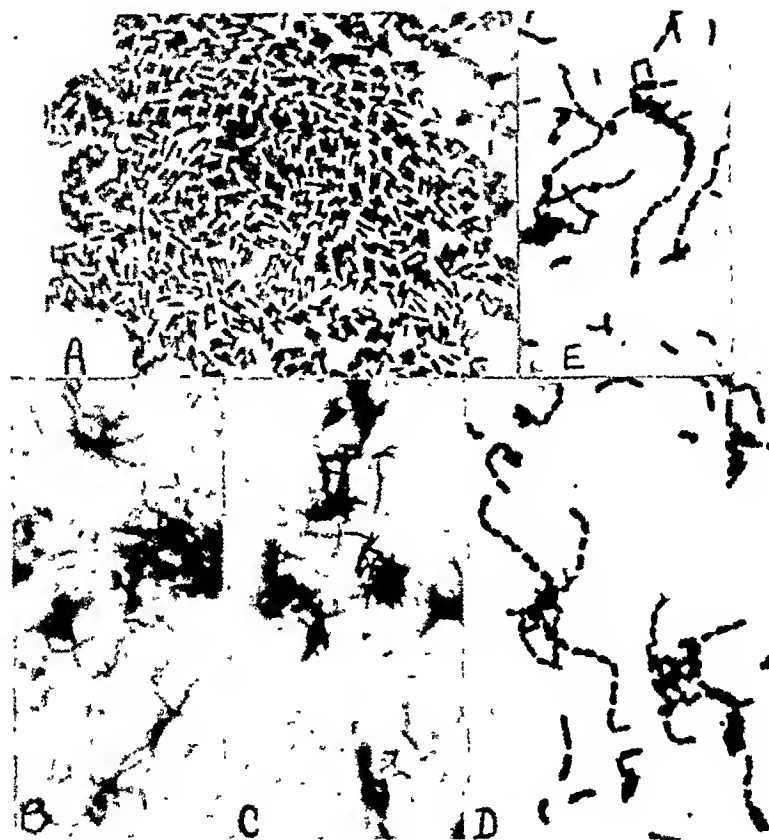


FIG. 3. MICROPHOTOGRAPHS OF BACTERIA

(a) Diphteroids from the original blood agar cultures, (b) bacilli in a section of the vegetation of the mitral valve, (c) bacilli in a smear from the vegetation of the mitral valve and (d) and (e) streptococcic forms from plain broth.

bacilli were present. These organisms were typical diphteroids showing palisade arrangement and no evidence of chain formation was present. They were straight, solid staining Gram positive rods of fairly uniform length with slightly rounded ends. On Loeffler's medium only solid staining forms were found. Original broth cultures showed organisms of similar character except that occa-

sionally they were arranged end to end, and on the whole were somewhat larger than those in the blood agar cultures, and occasionally filamentous forms like those seen in the sections of the heart valve were present. Smears of transplants made from single colonies of blood agar cultures into infusion broth, brain dextrose broth, and potato phosphate broth, as suggested by Callow, showed the organisms growing nearly exclusively in chains of bacillary forms, most of which resembled in size and shape the bacilli in blood agar cultures. Some long filaments were found and also some chains showed rounded forms indistinguishable from streptococci. Occasional chain showed part of the chain to be made of bacilli and parts to be streptococci in form. In some bacilli two densely staining ovoid or almost spherical forms could be seen, surrounded by an almost invisible, faintly staining residue of the original bacilli. Transplants made repeatedly over a period of eleven months revealed the same findings as seen on the first transplant. It has been impossible to produce a pure streptococcus from the culture on any medium by short or long periods of incubation. As soon as the organism was transplanted back to blood agar, only long rods were produced and no streptococcic forms were seen. Some chains of bacilli were present, but palisade arrangement was chiefly produced. A culture made for us by Dr. Ralph Mellon from a single bacillus showed the same morphological and cultural characteristics as those of the cultures made by picking isolated colonies from the heart valve and blood cultures.

The organisms in broth culture produced acid and no gas in glucose, maltose, saccharose, salicin, but produced no change in mannite, inulin, levulose, raffinose and lactose. It produced no indol. Growth on human serum agar containing 1 per cent sugar showed identical fermentation reactions with glucose, lactose, saccharose, maltose and mannite, the only sugars planted.

The blood serum of the patient agglutinated the organism in broth cultures at a dilution of 1:5120, and a strain of *Streptococcus viridans* from root abscess of a tooth of another patient, in the same dilution. Serum from a normal person failed to agglutinate either of these organisms at 1:20 or higher dilutions. Several times animals were injected intravenously with large amounts of broth cultures with no untoward results. On one occasion the growth from six blood agar slants was washed off in a total of 8 cc. of salt solution. Intravenous injection of 1.5 cc. of this was made into a white mouse, 3 cc. into a rabbit, and 2 cc. given intracardially to a guinea pig and 1.5 cc. into a rat, intraperitoneally. All the animals showed no effects from the injection. Subcutaneous injection of larger amounts into white mice and a guinea pig likewise produced no infection.

SUMMARY AND CONCLUSIONS

A case of subacute bacterial endocarditis running a typical clinical course and showing typical post mortem findings is presented. From the blood on four occasions during life and from

the blood and heart valve after death a diphtheroid bacillus was recovered in pure culture. Smears and sections of the heart valve revealed large numbers of the same organism with no other organism present. The serum of the patient obtained after death agglutinated the organism in high dilution. On liquid media streptococcic forms were produced, but only bacillary forms were found on blood agar.

This case well demonstrates that diphtheroid bacilli in blood culture may be of vital importance and are not always simple contaminants. The entire clinical and pathological picture presented by this patient and the bacteriological studies point strongly to the view that the organism obtained is only a stage in the life cycle of a green producing streptococcus. It is recommended that all strains of diphtheroid bacilli obtained from human cases of infection be cultured on appropriate mediums in order to demonstrate their relation to streptococci.

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EDITORIAL

TUBERCULIN P. P. D. (PURIFIED PROTEIN DERIVATIVE)

In 1890, the world was advised of a new discovery by Robert Koch, the patient, painstaking bacteriologist. The new product, a preparation from tubercle bacilli, at first advocated for therapeutic purposes, was finally, in the early 20th century, established as a reliable diagnostic agent in tuberculosis by von Pirquet, Calmette, Mantoux, and others, for both animal and man. Its value as a diagnostic agent and especially for intelligent survey purposes in man is undenied at present.¹ In veterinary practice, it is now even more extensively used than in human practice. To the genius of Robert Koch must be credited the recognition of the protein nature of the active principle of tuberculin, although the tests used by him might be considered rather crude today and leave a suspicion of doubt because the pure active principle had not been prepared by him; yet his experiments can be repeated with identical results. Tuberculin O. T. (old tuberculin Koch), the original glycerol broth concentrate upon which tubercle bacilli had been grown, defied the best chemical efforts for obtaining the pure active principle and it remained for Long² to initiate studies in 1919 for preparing a non-protein synthetic medium suited for growing tubercle bacilli with which the problem could be studied chemically, and finally for Seibert³ in 1928 to prepare the active principle in crystalline form. To the chemist, the preparation of crystals assured chemical purity, and so tuberculin was proved to be a pure crystallizable substance of protein nature. At the same time Seibert³ devised a method for preparing a standard undenatured tuberculin of any desired strength and presented a method of chemical assay, which made for more exact chemical production and disposed of elaborate and extensive biological testing as a guarantee of uniformity. Then arose the practical question of the advantages, if any, of

the chemically pure tuberculin as compared with the time-honored O. T. so universally used and accepted by the medical and veterinary professions. Tuberculin T. P. T.⁷ (meaning tuberculin, protein precipitated by trichloroacetic acid) solutions were found to be relatively stable and stock solutions of any strength, equal to or greater than that of O. T., could be prepared. Preliminary clinical tests indicated that 0.0001 milligram or less of T. P. T. will produce a skin reaction in tuberculous patients, and it is a safe and satisfactory product for diagnostic purposes. Human tests with the pure tuberculoprotein have been variously carried out, and, because of slight modification in preparation by the different commercial firms coöperating in this endeavor, the tuberculoprotein has been given different names. Thus, "M A 100" is one designation used in the Johnson chart for chemical analysis of the tubercle bacillus according to numbers assigned to Mulford and Company. Mariette and Fenger⁴ conclude that M A 100 human protein is as sensitive and as selective as O. T., and probably more so, and the initial and repeat doses are safe and large enough to pick out the majority of tuberculous individuals. They believe it a better testing substance than O. T. because of its purity and the ability to weigh it accurately. Lichtenstein² used T. P. T. obtained from Seibert on 944 tuberculous adults and found 97.7 per cent reacted to a diagnostic injection of 1:1,000 dilution, starting with a 1:1 dilution consisting of 10 mgm. per cubic centimeter, which is equivalent to undiluted O. T. (Parke, Davis & Co.). He believes it a reliable form of tuberculin for intracutaneous testing, and found skin sensitivity to diminish as the lesion becomes more extensive, as the symptoms become more intense, and with duration of the disease. More recently, the commercial product has appeared as P. P. D. (purified protein derivative) (Parke, Davis & Co. and Sharp and Dohme) in tablet form in milk sugar. The tablets are available in two strengths, for the first test 0.0002 mg. and for the second 0.05 mgm., which is dissolved in 1 cc. of buffer diluent supplied with the tablets, thus assuring the use of fresh solutions of the pure substance responsible for the specific reaction. Although it is only contended that the pure substance is as sensi-

tive and as selective as O. T. by those who have clinically compared the materials, it must be admitted that the presence of minute amounts of extraneous protein in O. T. should recommend the use of the pure tuberculo-protein for diagnostic purposes. Its therapeutic use has not been stressed commercially, or in scientific publications, up to the present; and although there appears to be no obvious obstacles to the therapeutic use of the pure tuberculo-protein, it may be well to await further studies and instructions before proceeding in the case where small differences may mean profound effects, especially in such a place as the eye.

—H. J. CORPER.

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NEWS AND NOTICES

MINUTES OF 1934 MEETING OF AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS IN CLEVELAND, OHIO

The business meeting was called to order by President Foord on Sunday evening, June 10th at seven o'clock in the Empire Room of the Hotel Cleveland. The minutes of the previous meeting were approved as published in the official JOURNAL. Reports of Committees followed.

REPORT OF SECRETARY-TREASURER

As indicated in the circular communications sent out to the membership your officers have devoted a great deal of thought to questions of economic and scientific aspects. Some of these questions will be presented to you for discussion by various Committees. I wish to call to your attention particularly a problem which needs to be solved and that is the pernicious practice of hospitals in the field of clinical pathology. During the past year the radiologists and anesthesiologists have brought this problem to the attention of the American Medical Association and various States. In some States the problem has already been solved under the medical practice act, such as in California and Indiana. The Attorney-Generals have ruled that anesthesia should be given by physicians and not by laymen. The Council on Radiology has prepared a resolution which will also be read at this meeting and which so far has received the endorsement of the American Medical Association.

Your Secretary is very glad to report that the membership has responded nobly in paying dues as by June first 321 members had paid their dues in full out of a total of 390. Two members have been dropped for non-payment of dues, three have resigned and four have died. The appended financial report reveals that the Society still has been able to operate without cutting into the surplus. Please note that the income exceeds the expenditures by only \$859.00 and \$520.00 of this represents application fees, some of which may have to be returned. Further, that the membership receives in the JOURNAL 63 per cent of their ten dollars. It is obvious that we are operating on a very close budget.

Your Secretary is very grateful to the membership for the coöperation accorded him particularly by the various Committees and officers of the Society.

A. S. GIORDANO, *Secretary-Treasurer*.

Motion made and seconded for acceptance of report. Carried.

FINANCIAL REPORT OF SECRETARY-TREASURER

Assets

<i>Balance on Deposit</i> —City National Bank.....			\$2,625.97
<i>Investments:</i>			
Commonwealth Edison Company #41022 4 per cent First Mtge. Gold Bond Series F-1981.....	\$1,000.00	\$945.00	
American Telephone & Telegraph Co. #70378 5 per cent Coll. Trust Gold Bond 1946.....	1,000.00	1,025.00	
City National Bank, Cert. #8 28 Shares Common Capital Stock.....	280.00	336.00	2,306.00
<i>Furniture and Fixtures</i>		\$601.75	
Reserve for Depreciation.....		601.75	
<i>Balance on Deposit</i> —Citz. National Bank in Liquidation by Receiver.....			655.38
<i>Total assets</i>			\$5,587.35

Motion made and seconded for acceptance of report. Carried.

REPORT OF BOARD OF REGISTRY

In our annual report for the year from May 1, 1933 to April 30, 1934 inclusive, we are gratified to record a continuation of the good progress of the Registry of Technicians. For the first time in our brief history, applicants were required to take an examination before being granted a certificate of qualification. These tests which are held semi-annually were conducted simultaneously in various cities of the United States and Canada, the first in October 1933 and the second in April 1934. They consisted of a practical and a written part, the questions having been prepared by a member of our Board.

At the October examinations sixty-seven applicants participated, of which number, fifty-six passed successfully. In the April tests there were 128 candidates. The results are not available at the time this report is written. At the first national examination we had thirty examiners, while during the recent one, forty-nine took part. We take the opportunity here to express our thanks to the colleagues who have given freely of their time and labor in furtherance of our cause to raise the status of the laboratory technician. Their services were entirely gratuitous, often at a personal sacrifice and carried out the traditional spirit of our profession in the interest of humanity.

During the past year, 288 registrants were added to our rolls, of which 251 received the designation of Laboratory Technician and thirty-seven were awarded the appellation of Medical Technologist. The total number of registrants at the end of our fiscal year is 2237, comprising a large fraction of the best technicians in the United States and Canada.

As in the previous years, we have been greatly assisted in this work by the hearty endorsement and coöperation of the American Medical Association, the American College of Surgeons and the American Hospital Association.

During the past year the work of the Registry was made known to wider circles of the medical profession and hospital administrations by interesting graphic exhibits held at the National conventions of the American College of Surgeons at Chicago, the Southern Medical Association at Richmond, Virginia, and the American Hospital Association at Milwaukee. These exhibits were designed and personally supervised by Dr. Roy R. Kracke, a member of our Board who was aided in the respective localities by Fellows of the American Society of Clinical Pathologists.

A major concern of the Registry is the supervision of training of technicians. It has been the endeavor of the Board to bring the teaching progressively up to the highest level commensurate with sound knowledge and efficiency of the students. To this end certain standards have been set up which have been gradually elevated from year to year as experience dictated. We do not approve any schools organized for profit and are encouraging the affiliation of training schools with universities and colleges of learning, always of course, under the direction of a competent clinical pathologist. At present students must have had a year's course in college chemistry and biology prior to entering training. A model curriculum for training schools has been set up by Dr. Asher Yaguda and is now awaiting approval and amendments by the Board prior to publication.

A very gratifying feature of the supervision of training schools is the valuable help and coöperation accorded us by the Council of Medical Education and Hospitals of the American Medical Association. Dr. William D. Cutter, the Secretary, has been conferring with our Board and has generously lent us the services of the Council's field men to inspect training schools in the course of their periodical inspection of hospitals for the American Medical Association.

The finances of the Registry, we are happy to state, are in a satisfactory condition due to the economy practiced and the fact that the members consider it a privilege to serve without compensation in this altruistic function of our Society. To insure safety of our funds all reserve above current expenses has been invested in Government Bonds and both principal and interest are held in trust by a trust company subject to the disposition of the lawful chairman and two members of the Board membership. A detailed financial report with audit by a certified public accountant has been transmitted to the Executive Committee. A summary of the financial report is herewith appended.

The office work of the Registry has grown to enormous dimensions, involving a vast correspondence with registrants, applicants, clinical pathologists, physicians, hospitals and seekers for information. It has necessitated additional equipment and hiring of extra help.

The complexity and organization of the Registry office is now in such a state of development that continuity is highly desirable and a change to another location would be fraught with difficulties.

The Board desires to express its appreciation of the valiant and efficient work done by the Registrar, Mrs. Anna Ruth Scott, who has endeared herself to all the registrants and to the members of the Board by the painstaking and tactful management of her arduous duties.

We thank the Fellows of our Society for their helpful coöperation with our Registry.

PHILIP HILLKOWITZ, *Chairman.*

FINANCIAL REPORT

Balance in Bank, May 1, 1934.....	\$5,208.50
Total Income, May 1, 1933 to April 30, 1934.....	5,185.72
Total.....	<u>\$10,394.22</u>
Disbursements, May 1, 1933 to April 30, 1934	8,095.28*
Balance.....	<u>\$2,298.94</u>

* Includes purchase of \$3,000.00 U. S. Government Bonds.

Motion made and seconded for acceptance of report. Carried.

REPORT OF COMMITTEE ON PUBLIC RELATIONS

The work of your Committee on Public Relations for the past year has been of two kinds, clerical and deliberative.

The clerical work has consisted of the compiling of information submitted by the Society's Counselors. We have prepared a list of physicians in the United States who are making tissue diagnoses and from it we can ascertain the distribution of this service. Maps showing this distribution have been prepared to furnish visual aid in the study of the situation. We also have information from twenty States as to the legal relation of clinical laboratories to the practice of medicine. This in most cases consists of the opinion of an attorney concerning the effect of the State's Medical Practice Act.

Our list of tissue pathologists includes more than 860 physicians who are reported by the Counselors of the American Society of Clinical Pathologists to be making diagnoses of tissue. Some of the State lists include only those who are doing laboratory practice exclusively, while others also include internists or surgeons who undertake tissue diagnosis in connection with their clinical work. No concerted attempt has been made to gauge the ability or experience of the individuals listed.

In accord with the spirit of the times, we must consider the question of certification of tissue pathologists. Opinions differ concerning how this should be done, or whether it should be done. No method will be entirely satisfactory. One essential thing is to encourage adequate early training and frequent post-graduate study. The latter is indispensable for those whose routine material is limited in amount.

The question has already arisen as to whether a hospital has the right to employ a pathologist on a fixed salary and make charges to patients for his services. This common practice is apparently illegal in some States and under attack in others. If the disturbance becomes general, some hospitals will inevitably solve the problem by ceasing to employ pathologists. Others will join the ranks of those small or poorly financed institutions which are now using members of their clinical staffs as laboratory supervisors without salary. As an economy measure this may perhaps be better than the employing of an unsupervised technician. The tendency for such supervision to become purely nominal, however, makes the practice a questionable one on which to base the approval of a hospital-grading body.

It does not seem unreasonable to hope that every approved hospital of 50 beds or more will sometime be required to have a competent clinical pathologist in charge of its laboratory. The size and type of service will determine how much of the pathologist's time is needed.

In general the pathologist has come to consider an adequate salary a satisfactory method of compensation. If this arrangement is found to be contrary to law, or at variance with public and professional welfare, it may be possible for the pathologist to rent from the hospital its laboratory facilities and conduct them as a private diagnostic service.

The hospital roentgenologists have a somewhat similar problem and the American College of Radiology has recently (February 13, 1934) passed resolutions looking toward a remedy. There is appended to this report a somewhat similar set of resolutions, for the consideration of the Society at this meeting.

We cannot and should not get away from the consideration of the economic problems which are peculiar to the practice of clinical pathology. New ones will come and go and the Society's influence will help solve some of them.

Right now there is a well-publicized sentiment against specialization in medicine. Excessive expense for non-essential service is claimed. It is inevitable for the true clinical pathologist to believe that modern medical science and practice needs and will continue to need his services.

The most urgent suggestions presented this year by the Society's Counselors have been these—

First, know more about laboratory measures and their use than do the clinicians who may call for your help, and

Second, figure out some way to make your skill available to and desired by your clinical associates.

The following resolution is presented in accordance with the recommendation and vote at the Round Table meeting, Friday, June 8th, 1934.

Whereas, it has come to the attention of the American Society of Clinical pathologists that certain practices are now in operation in many localities, adversely affecting the practice of clinical pathology and the best interest of the patient, and

Whereas, The American Society of Clinical Pathologists wishes to support the

resolutions of the American College of Radiology made under date of February 13, 1934 that it is the function of the physician to furnish medical care and of the hospital to furnish hospitalization, and

Whereas, Clinical Pathology is a medical specialty, comprehended in the practice of medicine and recognized by the American Medical Association, and

Whereas, The principle is recognized by the American Hospital Association, the American Medical Association and other national medical organizations that hospitals and other corporations or lay groups should not enter into the practice of medicine.

Therefore, Be it resolved by the American Society of Clinical Pathologists that all such practices are hereby condemned as prejudicial to the interests of both the medical profession and the laity, and contrary to sound public policy, and

Be it further resolved, That a copy of these resolutions be sent to the Council on Medical Education and Hospitals and to the Bureau of Economics of the American Medical Association, to the American Hospital Association, to the American College of Surgeons and to any other organizations as the Executive Committee may direct, requesting the support of the above named organizations in combating such practices.

C. W. MAYNARD, *Chairman.*

Motion made and seconded for acceptance of report. Carried.

REPORT OF SEROLOGIC CONFERENCE COMMITTEE

The desire of conducting a North American Conference on serologic methods in the diagnosis of syphilis similar to those conducted by the League of Nations has been constantly in the minds of certain members of the Society. During Dr. Simpson's year as President, he personally stimulated a great deal of interest in the subject and his preliminary investigations on ways and means are in the hands of the present Committee. Immediately after becoming President, Dr. Foord asked me to form a Committee to investigate further the feasibility of such a Conference to be sponsored and conducted by the American Society of Clinical Pathologists. Dr. Simpson was asked to serve on this Committee. Following an appeal to Surgeon-General Cummings of the United States Public Health Service, Dr. Vonderlehr, appointed for special work in venereal disease control, became most actively interested in the project. His constant thought and efforts have been most valuable in outlining a specific plan for carrying out such a Conference.

His plan calls for the collection by the United States Public Health Service of 500 blood serum samples from known syphilitics, treated (400) and untreated (100), 500 serum samples from probable non-syphilitics, 100 serum specimens from lepers at Carville, Louisiana, 300 whole blood samples (serum not removed)

and, if possible, 150 cerebrospinal fluid samples. All of these to be partitioned and distributed as quickly as possible (by air mail to distant laboratories) to certain selected representative serologists who have developed individual methods. These to be tested by these serologists by their own methods in their own laboratories. After distribution of all samples, two months should serve as the time for collecting data from the serologists. Following this there would be a meeting in Washington, D. C. of the committee of five, two representatives of the Society, two recognized syphilologists and a United States Public Health officer to evaluate the data and make recommendations. Formal announcement of the results to be made at the regular meeting of the American Society of Clinical Pathologists and the release of the material in printed form by the United States Public Health Service.

The Committee has approved of the plan. An application for grant-in-aid was made to the National Research Council on March 10, 1934 for the sum of approximately \$3,000, the amount necessary to carry out such a Conference in addition to the very valuable aid amounting to nearly \$5,000 to be furnished by the United States Public Health Service. On May 23, 1934 this body announced its regrets at being unable to grant the request.

However, because of the continued interest of the Surgeon-General, Dr. Vonderlehr and other officers of the United States Public Health Service, I recommend that the Executive Committee approve in principle of the plan outlined, and that a Committee be granted power to continue to represent the Society and to carry out such a Conference if it seems possible to do so.

A. H. SANFORD, *Chairman*.

Motion made and seconded for acceptance of report. Carried.

REPORT OF COMMITTEE ON INVESTIGATION OF MOLDS

In response to a motion passed at the Milwaukee meeting of 1933, Dr. A. G. Foord has appointed an additional standing Committee on the Investigation of Molds. This Committee was requested to establish some scientific connection whereby any members could submit cultures for identification, and further to establish criteria for diagnosis of the ordinary fungus infections met with by the practicing clinical pathologist.

During the past year your Committee has investigated the field of fungus infection to the extent that it is apparent that it is impossible to have anyone to identify a given culture of any fungus that would establish proof of pathogenicity.

We have decided that a collection of findings proving growth and reproduction of pathogenic fungi can only be established by a collection by members of sufficient data to establish such criteria and the publication by this Society of such a collected study as a standard method of diagnosis of fungus diseases.

At present we can only present a report of progress and request the continued support of the Society in the collection of material for study.

F. M. JOHNS, *Chairman*.

Motion made and seconded for acceptance of report. Carried.

REPORT OF NECROPSY COMMITTEE

The Secretary of the Society sent out a questionnaire to the membership of the Society asking for data concerning the number of deaths, the number of postmortems and the situation in each locality concerning the obtaining of autopsies. To this questionnaire the Committee received ninety-six replies from pathologists and institutions. These have been tabulated according to States and some very interesting statistics and information has been obtained.

It is hoped that the activities of the Committee will be continued not only with the Society's membership but perhaps by securing coöperation with the American Hospital Association's Committee on Postmortems. By interesting that Society's membership we may be able to secure more complete statistics and secure information concerning many local situations, etc. At some future time then the Committee could publish the information, showing methods of coöperation between hospitals, doctors, undertakers, coroners, etc., that should be employed in order to improve the postmortem situation throughout the United States.

A. V. ST. GEORGE, *Chairman*.

Motion made and seconded for acceptance of report. Carried.

REPORT OF THE TUMOR REGISTRY COMMITTEE

Being the only member of this Committee attending the Annual meeting, this report is essentially a personal communication.

The Tumor Registry is attempting to collect rare, interesting and border-line cases for the ultimate purpose of loaning these slides in groups to members of the Society. In another year this loan feature should be in operation. I recommend that the Tumor Registry Committee attempt to arrange a scientific exhibit at the next annual meeting of this Society.

The Registry is, and will be for sometime, in an experimental stage. Many local slide registries are springing up and to these some of the members are doubtlessly contributing. One of the older, larger and well-known registries has been discontinued during the past year because of lack of funds. So far the expense of this Registry has been negligible. It has been somewhat of a disappointment to find that some of the larger institutions are either unwilling or officially opposed to registering cases. It is apparent that there are several obstacles in the path of the success of this Registry which can be overcome only by the interest, support and enthusiasm of the members.

One important obstacle is the geographic separation of the members of the Committee. The Registry has two important duties as soon as a case is received. The first is to make a final and definite classification of the purpose of filing and the second is to correspond with the sender informing him of the opinion of the Committee and the diagnosis under which the case is filed. A third might be added, which will become continually more important, and that is to follow each case until death or for several years. It is generally admitted that the pathologist should know more about the prognosis of malignant diseases and additional information of this nature can only be acquired through an efficient "follow-up" system. The consultation and diagnostic service which this Committee renders is of the utmost importance for its ultimate success. The majority of the cases submitted to any registry are sent in for an opinion. This feature of the Registry has been lax and correspondence has been delinquent. Reasons for this, I believe, are obvious. One suggestion to overcome this obstacle would be to substitute for the Committee, a director who is a tissue pathologist of international repute whose opinion would not be questioned. Such a man would probably not have sufficient time to devote. A more practical suggestion would be to appoint as a committee the members of the Society in one city or one comparatively small area. Such a group could meet once a week and discuss intimately any cases received. In this way an efficient consultation service could be maintained which I am convinced is most important. Such a registry should be allowed to remain in one locality for at least two or preferably three years and then change to another city or area. While it is in one locality it certainly should absorb the desirable material in that locality for several years back. I earnestly recommend to the President and Executive Committee that the Tumor Registry Committee in the future be so appointed.

I believe the Registry Committee must be aggressive and must contact the men who, by personal contact or through their publications are known to have desirable cases for registration. Most of those I have received to date have been the result of personal correspondence. More explicit directions for sending the material to the Registry will be mailed to all members in the near future.

OSBORNE A. BRINES, *Chairman.*

Motion made and seconded for acceptance of report. Carried.

REPORT OF NECROLOGY COMMITTEE

It is with a spirit of humility and sadness that the Necrology Committee submits the following report. During the last year four of our members have died.

Obituaries

Marian E. Parker died in Kalamazoo, Michigan, September 8, 1933 of pulmonary tuberculosis and poliomyelitis. She was born on April 4th, 1895 in New York; graduated from Syracuse University Medical School in 1926; was a

member of the Staff of Maybury Sanatorium at Northville, Michigan and in 1929 located in Kalamazoo where she practiced until her death.

O. J. West passed away in Seattle, Washington, September 17, 1933. A native of Oregon, where he was born in 1866, he graduated from the Willamette Medical School of Portland, Oregon in 1888. In 1900 he commenced pathology with Dr. Robert Zeit of Chicago in the Chicago Post Graduate School where he remained for almost ten years. Then he again answered the call of home and the West opening the first clinical laboratory in Seattle in 1910. Here he also served as director to several hospital laboratories including the Providence, and was pathologist to the Harborview Hospital for the last two years. His recreations were hunting, fishing, golfing and teaching.

April 5th, 1934, J. H. Litterer, age 41, died of heart disease in Nashville, Tennessee. Born in Nashville he successfully pursued his education in his home town, obtaining a Phi Beta Kappa key from the College of Arts and Sciences and a medical degree from the School of Medicine of Vanderbilt University. During the World War he served as a Captain in the U. S. Medical Corps.

Ohio State University was shocked on the morning of March 5th to learn of the sudden death from coronary thrombosis of Ernest Scott who the week before had successfully managed the College of Medicine Centennial. A native of the State of Ohio being born in Athens, Ohio in 1875 and closely connected with the University since his father is the only living former president, he graduated from Ohio Medical University in 1900. He taught at his Alma Mater and at Sterling-Ohio Medical College, joining the Ohio State faculty in 1910 where he was chairman of the Pathology Department and secretary of the college.

It is fitting that we stand a moment in bowed meditation in memory of those comrades who have preceded us to the Land of Everlasting Peace.

There are many heroes who live and die
Of whom we have never heard,
For this great big brawling world goes by
With hardly a look or word.

But one of the bravest and best of all
Of whom the list can boast,
Is the man who falls on duty's call,
The man who dies at his post.

He who battles disease when death draws near,
And faces his fate each day,
Yet strives to help and comfort and cheer
His comrades along the way.

Who goes his way while he yet may do
And smiles when he suffers most,
It seems to me is a hero true,
The man who dies at his post.

J. J. MOORE, *Chairman.*

Motion made and seconded for acceptance of report. Carried.

NEW BUSINESS

Motion made and seconded to accept the resolutions made by Dr. Maynard in his Public Relations Committee report. Carried.

The following resolution made by Dr. Hunter was accepted:

- a. That a Committee be appointed to investigate the feasibility and practicability of establishing life-memberships in the American Society of Clinical Pathologists.
- b. That the Committee investigate the practicability of the establishment of a fund for the purposes of promoting the interests of the American Society of Clinical Pathologists in particular and Clinical Pathology in general. Contributions to this fund may be made by members of the Society by direct cash payments, insurance policies taken out in favor of the Society, or as may be left to the Society by wills from the estates of deceased members.
- c. That the Committee bring in a report and recommendations concerning the establishment of life-membership and the special fund for consideration by the Executive Committee and the American Society of Clinical Pathologists at the next annual meeting.

Motion made by Dr. Rhamy that a liaison committee, carefully selected, be appointed to cooperate with the American Medical Association and other agencies on economic questions and to establish by law and otherwise that clinical pathology is a definite specialty of medicine, to the end that the practice of this branch of medicine by laymen and by corporate or lay institutions or organizations be controlled or prevented. Passed.

Resolution made by Dr. Rhamy that a special Committee be appointed to study and devise ways and means by which a registry for the certification of tissue pathologists could be established by the Society. Passed.

Motion made by Dr. Exton that the President appoint a Committee of as many as he deems fit to consider from every point of view the practicability of the Society publishing a work on Clinical Pathology. This Committee shall report their conclusions to the President and Executive Committee, who if they think the project advisable are hereby empowered to appoint the Editor and Subeditors in the various specialties, and make any necessary financial arrangements that will enable the preparation of the material to go forward without waiting for the next meeting of the Society. Passed.

REVISION OF CONSTITUTION AND BY-LAWS

As adopted at the meeting of the American Society of Clinical Pathologists, Cleveland, Ohio, June 10, 1934

CONSTITUTION

Article I—Name

This organization shall be known as the American Society of Clinical Pathologists.

Article II—Objects

The objects of this Society shall be: (a) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease; (b) to stimulate original research in all branches of clinical laboratory work; (c) to establish from time to time standards for the performance of various laboratory examinations; (d) to elevate the scientific and professional status of those specializing in this branch of medicine; (e) to encourage a closer coöperation between the practitioner and the clinical pathologist.

Article III—Membership

SECTION 1. The membership of this Society shall consist of (a) Fellows, (b) Associate, (c) Honorary, and (d) Corresponding Members.

SEC. 2. Fellows shall be graduates from recognized medical schools who have specialized in the practice or teaching of clinical pathology (the latter to be in a recognized medical school) for at least three years after graduation and who are devoting a major part of their time to this field. They shall be members in good standing of their county and/or state medical society and of the American Medical Association. For the purposes of this section Clinical Pathology shall be defined as that branch of the science and practice of medicine which consists of the application of pathologic anatomy, physiology, chemistry, parasitology and bacteriology to the diagnosis of disease.

SEC. 3. Associate members shall be graduates of recognized scientific institutions who have made such contributions to any of the sciences relating to clinical pathology and whose membership will so further the objects of the Society as to make them eligible for associate membership. Associate members shall pay the regular dues and have all the privileges of Fellows except those of voting and holding office.

SEC. 4. Honorary members shall have distinguished themselves by research or personal sacrifice in the cause of scientific medicine to warrant their recommendation for election by the Board of Censors. They shall have all the privileges of active members except those of voting and holding office. They shall be exempt from paying dues.

SEC. 5. Corresponding members shall be residents of foreign countries in

good ethical standing who have distinguished themselves in any of the branches of clinical pathology. They shall be exempt from paying dues.

Article IV—Officers, Members of Standing Committees and Terms of Service

SECTION 1. The officers of the Society shall consist of a President, a Vice-President, a President-Elect and a Secretary-Treasurer. The President and Vice-President shall serve for one year. The President-Elect shall enter upon the duties of President at the annual meeting following his election. The Secretary-Treasurer shall serve for three years.

SEC. 2. The Standing Committees of the Society shall be an Executive Committee; a Board of Censors and a Board of Registry of Technicians.

SEC. 3. Officers and members of Standing Committees are to be proposed by the Nominating Committee or nominated from the floor by a Fellow of the Society and shall be elected by a majority of the votes cast at the annual business session.

SEC. 4. The Executive Committee shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected, two to be elected annually.

SEC. 5. The Board of Censors shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected, two to be elected annually.

SEC. 6. The Board of Registry of Technicians shall be composed of six Fellows who shall each hold office for three years or until their successors are elected, two of them to be elected annually.

SEC. 7. Officers and members of Standing Committees shall transfer promptly to their successors all funds, books, manuscripts, vouchers and other property of the Society on termination of their offices.

Article V—Meeting Place

The time and place of the annual meeting and other meetings of the Society shall be determined by the Executive Committee, notice of which shall be mailed to every Fellow at least thirty days prior to such meeting.

Article VI—Quorum

Twenty-five Fellows shall constitute a quorum.

Article VII—Amendments

This Constitution may be altered or amended by a vote of three-fourths of the Fellows voting at a regular meeting in executive session, provided said alteration or amendment had been submitted to the membership by publication or otherwise at least thirty days prior to the annual meeting.

BY-LAWS

Article I—Applications for Membership

Application for membership shall be made on a form authorized by the Society, signed by the applicant, recommended by two members and approved by the local Counselor and the Board of Censors. At least thirty days prior to the convention the Secretary shall send a list of applicants to every member of the Society.

Article II—Qualification for Membership

SECTION 1. Applicants for fellowship, associate and corresponding membership approved by the Board of Censors shall be elected by a Ballot of three-fourths of the Fellows voting at any regular meeting.

SEC. 2. Proposal for honorary membership may be made by a Fellow of the Society. Such proposal shall be made in writing and submitted to the Board of Censors. On recommendation by the Board such proposed member shall be elected as provided in Section 1.

Article III—Dues

SECTION 1. All Fellows and Associate Members shall subscribe to this Constitution at the time of their election to membership and shall pay an initiation fee of Ten Dollars (\$10.00), payable with the application for membership.

SEC. 2. The annual dues for Fellows and Associate Members shall be Ten Dollars (\$10.00), payable December first for the following year and if unpaid on January first the subscription for the official JOURNAL will lapse. Five Dollars (\$5.00) of the annual dues shall be used as subscription for the official JOURNAL. New members elected at the annual meeting shall pay dues for the current year of Five Dollars (\$5.00) to cover the subscription of the entire volume of the official JOURNAL for that year.

SEC. 3. Fellows in arrears for dues for sixty days shall be notified thereof by the Secretary-Treasurer by means of a "return receipt" registered letter. Fellows in arrears for ninety days shall be automatically dropped from the roll for non-payment of dues. Within one year after loss of membership for non-payment of dues, Fellows may be reinstated upon payment of all arrears and current dues.

SEC. 4. Resignation from the Society shall be submitted in writing to the Secretary-Treasurer who shall cause the same to be presented to the Executive Committee at the next annual meeting and the resignation shall not become effective until acted upon by the Executive Committee. No resignation shall be accepted from a Fellow or member owing dues.

Article IV—Duties of Officers and Standing Committees

SECTION 1. The President shall preside at all meetings of the Society. He shall appoint the Chairman of the Executive Committee and the Chairman of the

Board of Censors, be an ex-officio member of all committees and perform all other duties that devolve on him by custom and parliamentary usage.

SEC. 2. In the absence from any meeting of the Society of the President, the President-Elect and in the absence of both, the Vice-President shall perform the duties of President.

SEC. 3. The President shall appoint the members of all Special Committees enumerated in Article V of these By-Laws except those of the Board of Registry of Technicians. He shall also appoint any additional special committees ordered by the Society and shall be empowered to appoint such others as he may consider necessary and for which he has secured the approval of a majority of the members of the Executive Committee.

SEC. 4. The Secretary-Treasurer shall keep a correct and permanent record of the meetings and the transactions of the Society. He shall furnish a copy of this record to the Editor of the official JOURNAL for publication, conduct the correspondence and perform such other duties as pertain to the office of Secretary. He shall receive and be the custodian of the funds of the Society except the funds of the Board of Registry of Technicians which shall be held by the Chairman of that Board. Within thirty days following the close of the annual meeting he shall present a budget for the ensuing year which shall meet with the approval of a majority of the members of the Executive Committee. He shall incur no additional expense during the year without the consent of a majority of the members of the Executive Committee. He shall give bond satisfactory to the Executive Committee, the cost of which shall be borne by the Society. He shall make a complete financial report at the annual meeting of the Society. He also shall be ex-officio Secretary of the Executive Committee.

SEC. 5. The Executive Committee shall be the executive and administrative body of the Society during the interval between the regular annual meetings and shall be empowered to enter into contracts and authorize such expenditures as may be necessary to carry on the affairs and the business of the Society. Its actions always shall be governed by the Constitution and By-Laws of the Society. It shall audit the accounts of the Secretary-Treasurer as often as it deems necessary and the Chairman shall hold the bonds of the Secretary-Treasurer and the Chairman of the Board of Registry of Technicians. The Committee shall meet prior to the executive session of the Society. The Chairman shall prepare a report to be made to the executive session of the Society on its activities during the interval between the annual meetings and certify to the accounts of the Secretary-Treasurer and the Chairman of the Board of Registry of Technicians. The Committee shall meet also immediately after the annual meeting of the Society to transact such business as properly may come before it.

SEC. 6. The Board of Censors shall investigate all applications for membership and submit their recommendations at the annual meeting of the Society. They shall receive and consider all complaints concerning the conduct of members and present a report at the executive session with their recommendations.

Suspension or expulsion from membership in the Society shall be by three-fourths vote of those members present and voting at a regular executive session.

SEC. 7. The Board of Registry of Technicians shall elect its own Chairman and Secretary. It shall conduct a Registry of Technicians, receive applications for such, pass on their qualifications and issue certificates and renewals of certificates to those meeting the requirements. It shall investigate schools for the training of technicians and register those approved. It shall conduct a placement bureau for technicians. Within thirty days following the close of the annual meeting the Chairman shall present a budget for the ensuing year which shall meet with the approval of a majority of the members of the Executive Committee. He shall incur no additional expense during the year without the consent of a majority of the members of the Board of Registry. The funds of the Registry shall be held by the Chairman of the Board who shall give bond satisfactory to the Executive Committee, the cost of which shall be borne by the Registry. The funds of the Registry shall be used only for the activities of the Registry. The Chairman shall make a complete financial report at the annual meeting of the Society.

Article V—Special Committees and Editor

SECTION 1. A Board of Counselors shall be appointed by the President to serve for one year. They shall represent such districts as may be determined by the President. It shall be the duty of the Counselors to act in the interest of the organization in their respective districts.

SEC. 2. A Nominating Committee of three Fellows shall be appointed by the President at the opening of the annual session, whose duty shall be to prepare a list of nominees for the elective offices for balloting by the Society. Additional nominations may be made from the floor.

SEC. 3. The President shall appoint a Program Committee consisting of three Fellows to serve for one year, the Chairman of which shall be the Secretary of the Society, whose duty it shall be to arrange the scientific program for the annual meeting.

SEC. 4. The President shall appoint a Committee on Exhibits consisting of three Fellows, one of whom shall be the Secretary of the Society, to serve for one year, whose duty shall be to arrange for scientific and commercial exhibits at the annual meeting.

SEC. 5. The President shall appoint a Research Committee consisting of three Fellows, to serve for one year, whose duty it shall be to foster and direct collective investigation and to collect from rare or obscure conditions data and materials to be made available to Fellows for study.

SEC. 6. The Executive Committee shall appoint an Editor for the official JOURNAL of the Society to serve for a term of three years. The Editor so selected together with the President of the Society and the Chairman of the Executive Committee, shall appoint an Advisory Editorial Board to serve for a period of

three years. The duties of this Board shall be to foster and supervise all official publications of the Society.

Article VI—Awards

At each annual session the Research Committee may designate a Fellow of the Society to receive the Ward Burdick Award. This award shall be in the form of a gold medal which shall be presented to that Fellow who, in the opinion of the Research Committee, has presented the most meritorious contributions to the science of clinical pathology. Rules governing the award shall be made by the Research Committee, approved by the Executive Committee and published for the information of Fellows of the Society. If, in the opinion of the Research Committee, at any annual session no contribution is judged of sufficient merit to receive the award, no award shall be made at that session.

Article VII—Elections

SECTION 1. The Society shall elect annually by ballot at an executive session at the annual meeting the following officers and members of committees: President-Elect, Vice-President, two Fellows to fill vacancies on the Executive Committee, two Fellows to fill vacancies on the Board of Censors and two Fellows to fill vacancies on the Board of Registry of Technicians and such other vacancies as may have occurred. The Secretary-Treasurer shall be elected in the same manner each third year.

SEC. 2. Election shall be by a majority of votes cast by the Fellows present and shall be from nominees proposed by the Nominating Committee or from nomination made by any Fellow present.

SEC. 3. The President-Elect and newly-elected officers shall be inducted into office at the conclusion of the meeting.

Article VIII—Vacancies

In the event of a vacancy occurring in the office of President, the unexpired portion of his term of office shall be filled by the Vice-President. Vacancies occurring in the offices of Vice-President and Secretary-Treasurer shall be filled for the unexpired term of office by the Executive Committee. If a vacancy should occur in the office of President-Elect, at the next annual meeting the Society shall elect a President in addition to the officers enumerated in Article VII, Section 1 of the By-Laws. Interim vacancies occurring on the Executive Committee, the Board of Censors and the Board of Registry of Technicians shall be filled until the next annual meeting by the Executive Committee.

Article IX—Code of Ethics

SECTION 1. The Code of Ethics of this Society shall be the same as that of the American Medical Association.

SEC. 2. It shall be deemed unethical for members to publish objectionable

laboratory advertisements in any form whatsoever; the Board of Censors shall act as judges in the matter, the members having the privilege of appeal to the Society at a regular executive session.

SEC. 3. It shall be considered unethical for a member to lend his name for publication in any laboratory advertisement or announcement which violates the Code of Ethics. The borrowing of names of other physicians, scientists or laymen, on the basis of an occasional service or consultation, for purposes of advertising or to sanction the work of a laboratory is misleading and unethical.

SEC. 4. Any system of dividing or rebating fees for laboratory services shall be considered unethical.

Article X—Standing Rules

SECTION 1. The Chairman, at all regular annual meetings, shall first call the members assembled to order in executive session for the purpose of transacting such business and appointing such committees as are herein required, together with the making of other arrangements consistent with conducting the annual meeting.

SEC. 2. Scientific papers presented by Fellows shall be limited to twenty minutes; those presented by guests shall not occupy more than thirty minutes. A longer time may be granted only by the consent of a majority of the Fellows present.

SEC. 3. The opening discussion on each paper shall be limited to ten minutes; succeeding discussions shall be limited to five minutes each except as extension of time may be granted by a majority of the Fellows present.

SEC. 4. Members desiring to speak twice on any one subject must obtain the consent of a majority of the Fellows present.

SEC. 5. Non-members may be given the privilege of the floor only by consent of the majority of the Fellows present.

SEC. 6. A paper read before this Society becomes the property of the Society, to be published in the official JOURNAL provided it meets the approval of the Advisory Editorial Board, except that the privilege for prior publication may be granted by the Editor.

SEC. 7. Order of Business for Executive Session:

1. Call to order.
2. Reading of minutes.
3. Unfinished business.
4. Reports of committees.
5. Election of members.
6. New business.
7. Nominations.
8. Election of officers.
9. Induction of officers.
10. Adjournment.

Article XI—Parliamentary Procedure

All parliamentary proceedings at the meetings of this Society shall be governed by Roberts' Rules of Order, except where otherwise provided.

Article XII—Amendments

Amendments of these By-Laws must be submitted in writing at the opening of the annual meeting and shall be voted upon at the executive business session. A majority of the votes cast shall be required to amend.

THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS ROSTER FOR 1934

OFFICERS

Dr. Frederick H. Lamb.....	President
Dr. Benjamin S. Kline.....	Vice-President
Dr. Foster M. Johns.....	President-Elect
Dr. Alfred S. Giordano.....	Secretary-Treasurer

EXECUTIVE COMMITTEE

Dr. Alvin G. Foord, Chairman	Dr. H. C. Sweany
Dr. K. Ikeda	Dr. H. J. Corper
Dr. W. M. Simpson	Dr. C. I. Owen

PAST PRESIDENTS

1922-3	Dr. Philip Hillkowitz.....	Denver, Colorado
1923-4	Dr. Wm. Carpenter MacCarty.....	Rochester, Minnesota
1924-5	Dr. John A. Kolmer.....	Bala-Cynwyd, Pa.
1925-6	Dr. Frederic E. Sondern.....	New York, N. Y.
1926-7	Dr. Wm. G. Exton.....	New York, N. Y.
1927-8	Dr. A. H. Sanford.....	Rochester, Minnesota
1928-9	Dr. F. W. Hartman.....	Detroit, Michigan
1929-30	Dr. J. H. Black.....	Dallas, Texas
1930-1	Dr. K. M. Lynch.....	Charleston, S. C.
1931-2	Dr. H. J. Corper.....	Denver, Colorado
1932-3	Dr. Walter M. Simpson.....	Dayton, Ohio
1933-4	Dr. Alvin G. Foord.....	Pasadena, California

MEMBERS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

GEOGRAPHIC DISTRIBUTION

* Associate Members.
† Counsellors.

§ Corresponding Members.
** Honorary Members.

FOREIGN

**ACHARD, CHARLES.....	Academy of Medicine, Paris, France
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REGISTRY OF TECHNICIANS

In view of the encouragement given by the American College of Surgeons to have all technicians of approved hospitals apply for certificates from the Registry, a large number of new applicants have taken the October examinations.

At the meeting of the Board of Registry held in Cleveland, during the convention of the American Society of Clinical Pathologists, considerable business was transacted, the most important being

1. Examination of applications of 24 candidates for rating of Medical Technologist. Fifteen were rejected and nine approved.

2. Consideration of the applications of 16 training schools for approval and registration. Two of them were approved, ten rejected, and four were held over pending the joint action of the Board and the Council on Medical Education and Hospitals of the American Medical Association, which will be taken within the next year to unify the standards of hospital training course for laboratory technicians. The final action must necessarily await the completion of inspection of training schools throughout the United States by the inspectors of the Council.

3. Decision to publish in our official JOURNAL the examination questions and the names of those who successfully passed the examination.

4. Re-emphasis on the decision that no technician shall be granted a certificate of registration who operates a private laboratory "regardless of location."

5. Decision to the effect that after 1936 the minimum requirements for registration of laboratory technicians shall be raised to two years of college work including major sciences. The technicians who meet the minimum requirements of one year college including chemistry and biology and who shall have completed their training prior to 1936, are eligible to register after 1936. The credits submitted should be verified through the state board of education or other authorized educational agent before the student is enrolled.

An informal conference was held by the members of the Board and Doctor Cutter of the Council on Medical Education and Hospitals of the A. M. A. and members of his staff. It was decided to formulate the essentials for approval of training schools for laboratory technicians by the joint action of the Council and the Board as soon as inspection of a sufficient number of the training schools has been completed by the Council's representatives during the coming year. It is the desire of the Council to cooperate with the Board in this

undertaking and not to supplant the Board or duplicate the program of the Board in this matter. In the future, any action to be taken by the Board with reference to approval of training schools for laboratory technicians is to be referred to the Council for its approval and endorsement. Eventually approval of these schools will be granted only by the joint action of the Council and the Board, according to the minimum requirements or essentials to be formulated jointly.

The JOURNAL calls attention with a great deal of pride to the increase in advertising in its pages. It is a particular pleasure to call attention to the advertisements of the Corning Glass Company, Research Supply Corporation, Loeser Laboratories, Reiker Instrument Company, and Central Scientific Company. While some of these organizations have been advertisers in the past, they have recently renewed their contracts with the JOURNAL. Members of the Society will find these high class firms ready to supply them with good laboratory material. In ordering supplies from these and our other advertisers, we urge you to refer to the JOURNAL.

INDEX TO VOLUME 4

- A** **GRANULOCYTOSIS**, 453
 Alcohol, test, 182
 Allergic disease, 446
 Allergic state, 169
 ALT, H. L., 354
 Amebiasis, diagnosis, 470
 Antigen emulsion, 426
 Antigen, Wassermann, 235, 301
 Antitoxin, streptococcus, standardization, 200
 Appendix, carcinoids, 297
 Arsenic in brain, 362
 Artificial fever, 373
 Asphyxia, death by, 66
- B** **BACTERIOPHAGE**, 336
 BAKER, R. L.; OSGOOD, E. E., AND WILHELM, M. M., 292
 Ball test antigen, 426
 BAKES, H. M., 260
 BECK, R. C., 189
 Blood dyscrasias, 370
 Blood, iodine in, 346
 Book reviews, 318, 379
 BOYD, JR., D. A.; KING, W. E., AND CONLIN, J. H., 336
 Breast lesions, 327
 Brucelliasis, 312
 BUTLER, C. S., 239
- C** **CANCER** problem, 321
 Carcinoids, appendix, 297
 Children, reticulocytes, 292
 CONLIN, J. H.; AND KING, W. E., 200
 CONLIN, J. H.; KING, W. E., AND BOYD, JR., D. A., 336
 Constitution and By-laws, 519
 Convention, A. S. C. P., 314, 376
 CORPER, H. J., 504
 CURTIS, G. M.; AND PHILLIPS, F. J., 346
 Cutting, death by, 66
 Cyanide poisoning, 429
- D** **EXTROSE** tolerance test, 381
 Diagnosis, amebiasis, 470
 Diluting antigen, 301
 Diphtheroid, endocarditis, 492
 Drowning, death by, 42
- E** **EDITORIAL**, 239, 312, 373, 446, 504
 Encephalitis, arsenic in, 362
 Endocarditis, 492
- Etiology, granulopenia, 453
 EXTON, W. G., AND ROSE, A. R., 381
- F** **ECES**, iodine in, 346
 Fever, artificial, 373
 FOORD, A. G., 247, 321
 FOORD, A. G., AND STONE, W. J., 492
 Friedman test, 242
- G** **CASSING**, findings in, 309
 GETTLER, A. O., 50
 GETTLER, A. O., AND ST. GEORGE, A. V., 429
 GIORDANO, A. S., 312
 Granulopenia, etiology of, 453
- H** **EISE**, H. A., 182, 400
 Hemoglobin methods, 354
 Hemorrhage, brain, 362
 Hepatolienography, 212
 Hyperpyrexia reaction, 260
- I** **INTERMEDIN**, test for, 485
 Iodine in blood, urine, feces, 346
 Iso-agglutinins, medicolegal, 400
- K** **KERNOHAN**, J. W., 410
 KERNOHAN, J. W.; AND OSTERBERG, A. E., 362
 KING, W. E. BOYD, JR., D. A., AND CONLIN, J. H., 336
 KING, W. E., AND CONLIN, J. H., 200
 KLINE, B. S., 426
 KOLMER, J. A., 301
 KOLMER, J. A., AND RICHTER, C. E., 235
 KRACKE, R. R., AND PARKER, F. P., 453
- L** **AMB**, F. H., 327
 Lipoids, Wassermann antigen, 235
 Liver extract, in neutropenia, 438
- M** **ALIGNANT** neutropenia, 438
 MARKOWITZ, B., 370
 MARTIN, W. B., 438
 MARTLAND, H. S., 66
 MATZ, P. B., 309
 Medicolegal necropsy, 1, 24, 32, 50
 Medicolegal system, 7
 Methods, hemoglobin, 354
 Methods, iodine, 346
 MILOSLAVICH, E. L., 42

Minutes, thirteenth annual convention, 507

NASAL sinuses, allergy, 169
 Necropsy, committee report, 161
 Necropsy, medicolegal, 1, 24, 32, 50
 Necropsy, toxicologic, 410
 Neutropenia, 189
 Neutropenia, treatment of, 438
 Norms, C., 24

OSGOOD, E. E. BAKER, R. L., AND WILHELM, M. M., 292
OSTERBERG, A. E., AND KERNOHAN, J. W., 362

PARKER, F. P.; AND KRACKE, R. R., 453
 Passive transfer in allergy, 446
 Pathologist's rôle, 321
 Pathology, psittacosis, 247
PHILLIPS, F. J., AND CURTIS, G. M., 346
 Poisoning, cyanide, 429
 Pregnancy tests, 242
 Presidential address, 321
 Program, A. S. C. P., 314
 Psittacosis, 247

REACTIONS, intravenous therapy, 250
 Reactions, bacteriophage, 336
 Red bellied dace test, 455
 Respiratory tract, allergy, 169
 Reticulocyte counts, 232
RICHTER, C. E.; AND KOLMER, J. A., 235
ROSE, A. R.; AND EYTON, W. G., 381

ST. GEORGE, A. V., 32, 297
ST. GEORGE, A. V.; AND GETTLER, A. O., 429
SCHULTZ, G. T., 7

Shooting, death by, 66
SHUSHAN, M.; AND TRIPOLI, C. J., 470
SIMPSON, W. M., 374
 Skin test, 209
 Society news and notices, 212, 314, 376, 450, 507
 Society roster, 527
SONDERN, F. E., 1
 Stabbing, death by, 66
 Staphylococcus bacteriophage, 336
STEINBERG, B., 169
 Stool cultures, ameba, 470
STONE, W. J.; AND FOORD, A. G., 492
 Symposium, medicolegal necropsy, 1
 Syphilis and yaws, 239
 System, medicolegal, 7

TECHNIC, neurologic, 410
 Test, alcohol, 182
 Test, dextrose tolerance, 381
 Test, intermedin, 485
 Thorium dioxide, 212
 Thorotrast, 212
 Toxicology, medicolegal, 50
 Toxins, streptococcus, standardization, 209
TRIPOLI, C. J., 212
TRIPOLI, C. J., AND SHUSHAN, M., 470
TUBERCULIN, P. P. D., 504

URINE, iodine in, 346

VAUGHAN, W. T., 446

WARFARE gassing, 309
 Wassermann, antigen, 235, 301
WILHELM, M. M.; OSGOOD, E. E., AND BAKER, R. L., 292

YAWS and syphilis, 239
YOUNG, A. M., 485

